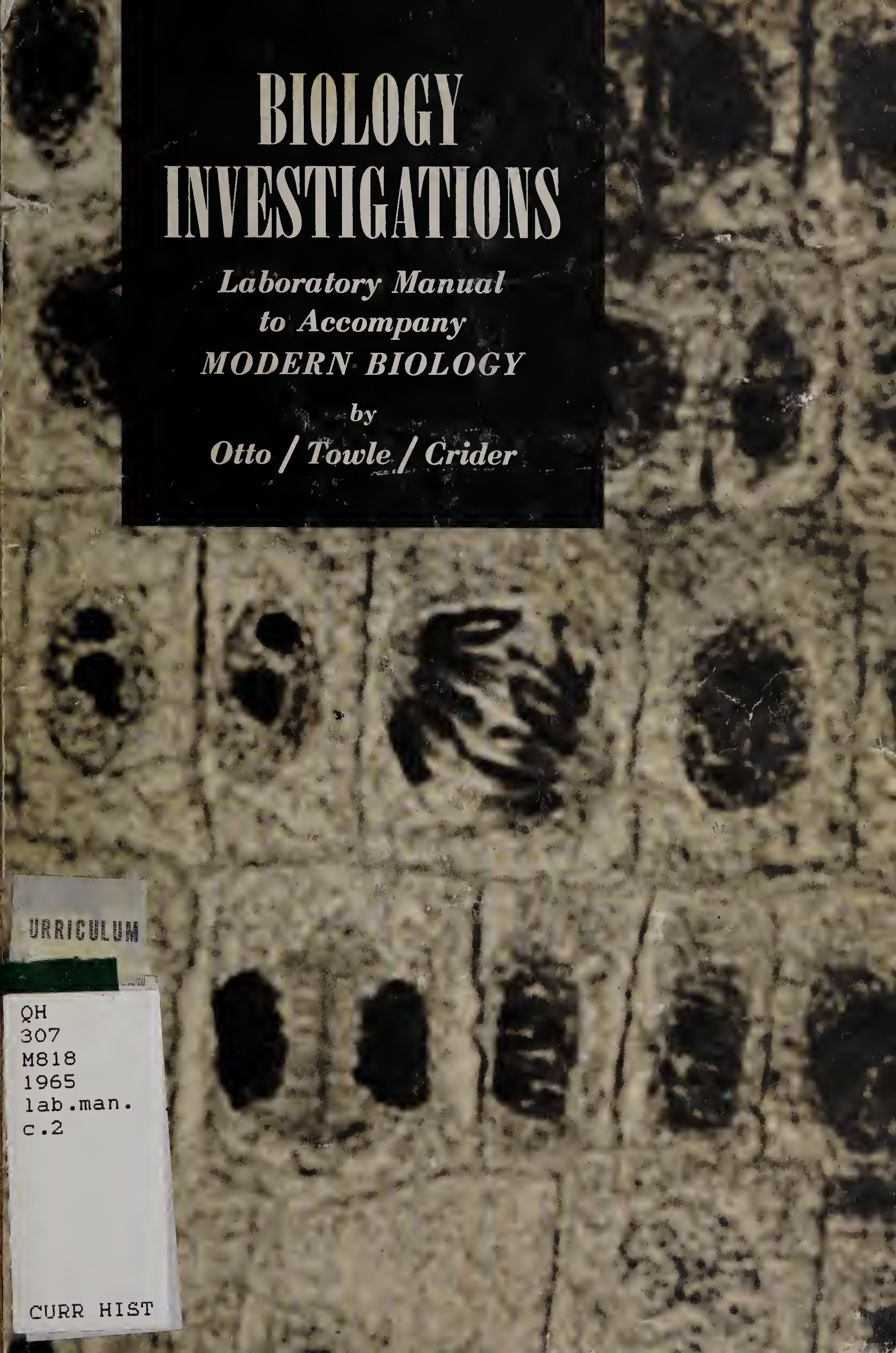

BIOLOGY INVESTIGATIONS

Laboratory Manual
to Accompany
MODERN BIOLOGY

by
Otto / Towle / Crider

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BIOLOGY INVESTIGATIONS

Laboratory Manual
to Accompany
MODERN BIOLOGY

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Holt, Rinehart and Winston, Inc., New York

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Preface

The laboratory phase of the biology course is receiving increased emphasis today. As a supplement to the textbook in the biology program, BIOLOGY INVESTIGATIONS provides the student opportunity to add his own experiences and discoveries to the body of biological knowledge presented in his textbook in a well-rounded biology program.

This edition of BIOLOGY INVESTIGATIONS offers a laboratory program supporting all major areas of biology included in MODERN BIOLOGY. This broad scope of laboratory experiences puts an investigative emphasis on all parts of the course. Thus the biology course is a laboratory science and is not merely a science course with selected laboratory experiences which may or may not correlate with areas of study in the textbook.

Areas of increased emphasis in this edition of BIOLOGY INVESTIGATIONS reflect current achievement and advance in biological science today. Among these areas are biochemistry, molecular biology, cell biology, genetics, microbiology, physiology, and ecology. Increased emphasis has been placed on observing, recording, and evaluating experimental data. Background information is given for each exercise, and the student is expected to conclude with an understanding of the biological significance of his experiment.

Much use is made of living materials. At the same time, BIOLOGY INVESTIGATIONS remains basically teachable. Each Investigation has been classroom tested many times. Each one is workable in the high school biology laboratory with supplies and equipment usually at hand or readily available. Directions are sufficiently detailed to permit the student to obtain meaningful results through his own efforts, with a minimum of teacher supervision. It is hoped that the laboratory program as presented in BIOLOGY INVESTIGATIONS will give the student a *personal experience* to make his endeavors meaningful and his learning permanent. *Critical observations, accurate recording, evaluation,* and *interpretation of data* and *inductive reasoning* in discovering principles and developing concepts are emphasized throughout the laboratory program.

Each Investigation is divided into Parts of varying difficulty. This division provides great flexibility in adjusting the laboratory program to the time available, equipment and apparatus on hand, and degree of emphasis of each area and topic in the course. It also provides for student ability groupings and individual differences in members of a class. Parts designated as A-level are basic laboratory procedures for which specific directions for conducting the work and recording observations are provided. Most of these Parts are planned for individual laboratory study conducted simultaneously by all members of the class. These basic studies represent the core of the laboratory program and correlate closely with MODERN BIOLOGY.

The teacher may wish to develop certain topics in greater depth or provide additional opportunity for individuals or small groups of students to pursue more advanced study. B-level Parts supply this need. In these Parts, procedures and techniques are more advanced and the student is required to interpret his experimental data. While the student is left more on his own resources in working at this level, he is given sufficient direction to proceed independently.

The highly interested and motivated student, capable of performing open-ended study will find abundant suggestions in Parts designated as C-level. These Parts are intended for extra-class investigation extending over a period of several days to several weeks. Students are expected to plan their experiments or field studies and use their own resources and ingenuity in recording observations, compiling data, and drawing valid conclusions. Suggestions for materials, supplies, and equipment needed as well as for procedure and presentation of results are provided as a means of encouraging more capable students to engage in study at this level with a minimum of individual direction by the teacher.

A set of Tests and a set of Alternate Tests are included in the MODERN BIOLOGY Program. When feasible, conceptual questions designed to stimulate critical thinking and data analysis are included. One set of tests is sent with each copy of BIOLOGY INVESTIGATIONS. The teacher may specify the test series desired.

The TEACHER'S GUIDE TO THE MODERN BIOLOGY PROGRAM includes extensive professional material for each laboratory Investigation with answers to questions, in addition to suggestions for teaching the textbook and the Key to Tests and Alternate Tests.

The authors of BIOLOGY INVESTIGATIONS are indebted to those teachers who prepared *Laboratory and Field Studies in Biology: A Sourcebook for Secondary Schools* (Edited by Lawson and Paulson—Holt, Rinehart and Winston, 1960), from which several Investigations in this edition have been adapted. In addition, they would like to express their appreciation to H. DuBuy and J. Showacro for information gained from their paper, Laboratory Experiments in *Biology, Physics and Chemistry* (mimeographed edition, National Institutes of Health, cited in *Science World,* December 12, 1962), and adapted for use in Part 3 of Investigation 4-1.

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Working As A Biologist

Conducting a biological investigation. As a biology student you will spend much of your time in the laboratory discovering from actual materials the details of the structures and functions of living things and their relationship to one another. You will learn to use some of the methods used by scientists: experimentation, observation, collection of data, interpretation of observations and results, and drawing of conclusions.

Each Investigation is planned to increase your ability to use laboratory apparatus, improve technique, find scientific answers to many problems, and increase your knowledge of living things. Each Investigation begins with a statement of its purpose and background information related to it.

Because the Investigations usually include several distinct activities, they are divided into related parts: 1, 2, 3, and so on and in many Sections within parts. Your teacher will tell you whether you will be expected to do all Parts or only certain ones. The length of time required to complete each Part will vary with the nature of the Investigation. In some you will be asked to follow an outlined procedure; others will involve experimentation on your own.

Recording biological data. Biological data include all observations you make during an Investigation. The data you record will be in several forms. In those Investigations dealing with *structure* (the parts that form a living thing), you may be asked to record your observations by labeling a drawing that has been provided. More frequently you will be asked to provide the drawing as well as the labels. Biological drawings are labeled by running a guide line from the part to be designated to the *printed* name of the part. Names of parts are printed horizontally, never vertically.

You may be asked to *sketch* a particular organism. The sketch is used to show the general shape and approximate size and location of the important organs or structures. For example, four sketches are shown in Fig. 29-1-a on page 257 of this book.

Most of the drawings you will be asked to make are *representative drawings.* These show the exact size, shape, and locations of structures found in an organism. Fig. 38-1-a on page 290 of this book is an example. The drawings you make yourself need not be artistic; few of us are artists. But they *must be neat and accurate or they have no scientific value.*

A *diagram* is used to represent relationships. It does not have to be definite. Fig. A is a diagram of the inheritance of color blindness in humans. A woman who carries a gene for color blindness marries a man who has normal color vision. They have six children—four sons and two daughters. You can study the key to the symbols and determine the percent of sons who are color blind and the percent of daughters who are carriers of the trait. The study of the diagram also reveals the manner in which the trait is inherited.

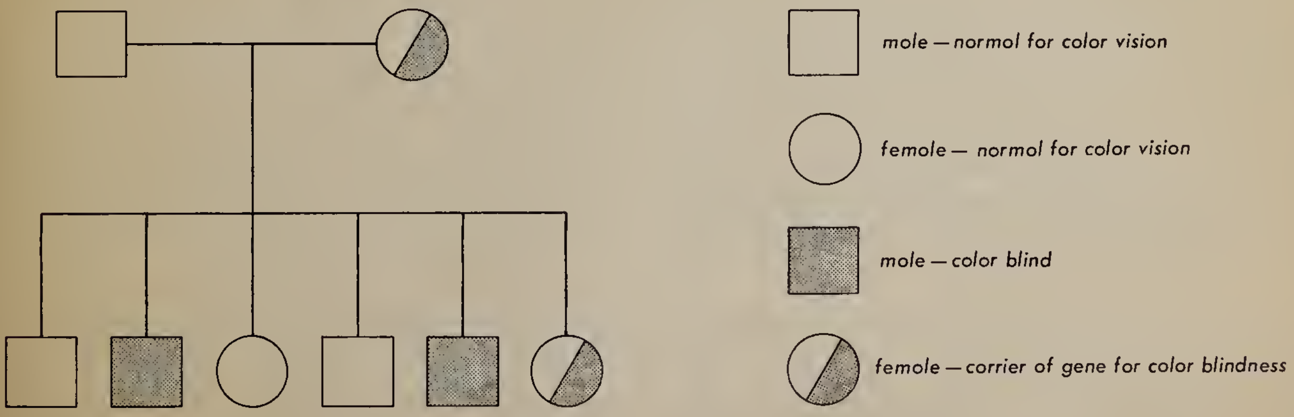

male — normal for color vision
female — normal for color vision
male — color blind
female — carrier of gene for color blindness

Fig. A Family history illustrating the inheritance of color blindness in man

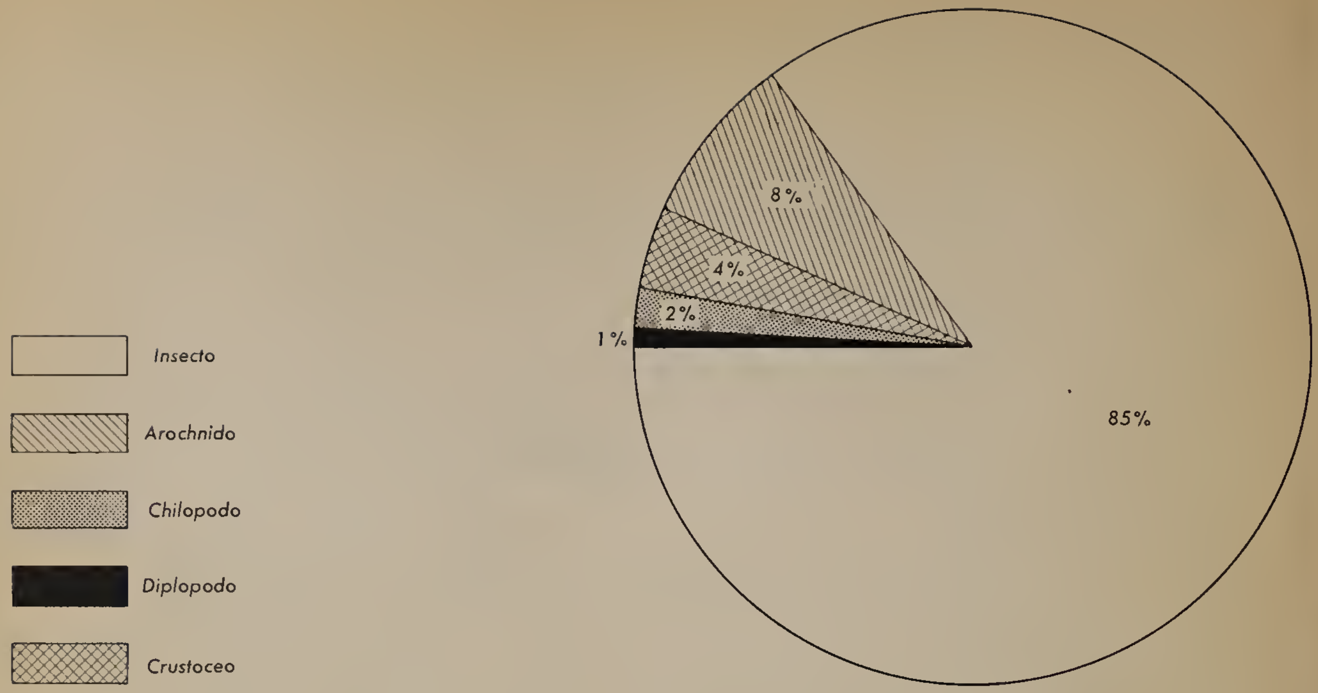


Fig. B A circle graph representing the kinds and percent of arthropods found by the members of a class

Data can also be interpreted by showing relationships between two or more variables in graphic form. For example, members of a class were told to bring in representatives of the phylum Arthropoda. Of the total number of representatives brought in 85% were insects (Insecta), 8% were spiders and their relatives (Arachnida), 2% were centipedes (Chilopoda), 1% were millepedes (Diplopoda), and 4% were crayfish and their relatives (Crustacea). The *circular graph* can be used for tabulating the results of this study. Fig. B shows the relative number of representatives of arthropod classes found by members of the class in a population study in a given area.

The *bar graph* is a second method of representing data. A student has done an experiment in which he studied the effect of various solutions on the heartbeat of the water flea. He used the natural habitat in which the water flea had been living for his control. He found that the heartbeat at a rate of 130 times per minute when the animal was left in its normal aquatic surroundings. Fig. C contains the data relating to the rate of heartbeat when the water flea was transferred to another medium.

We can also represent data by using a *line graph*. A class studied the effect of temperature on the respiration rate of the goldfish as indicated by movements of the gill cover.

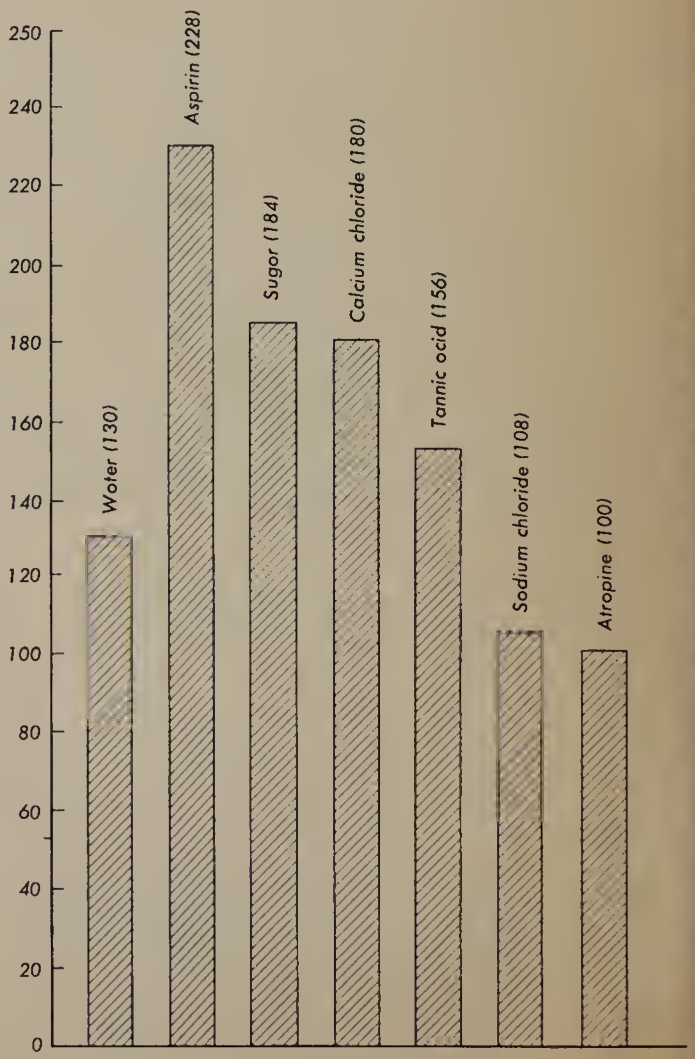


Fig. C The effect of various solutions on the heartbeat of *Daphnia*

After studying several goldfish and tabulating the movements of the gill cover per minute in a number of goldfish, the class had the following results:

Temperature	Average movements of gill cover/minute
32°F	0
37°	13
42°	20
47°	25
52°	30
57°	35
62°	43
67°	49
72°	58
77°	81
82°	104
87°	120
92°	133

In this experiment there were two variables, temperature and movements of the gill cover per minute. Fig. D is a line graph of the results, showing the effect of temperature on the respiration rate of the goldfish.

If the subject of the Investigation deals with the *behavior* of living things (how they react and what they do) or the *function* of an organ (what it does to keep the organism alive), your observations will usually be in the form of answers to direct questions, data to record, or tables to complete. These answers should be based on scientific facts, gained from your own observations as well as from your reading. Guesswork must *never* appear in scientific study. Although the results of the Investigation or your own experimentation are not always what you would expect, remember that *often the experiment that doesn't work is just as important to research as the one that does.*

Preparing a scientific write-up. Writing a scientific report on individual experiments is very different from making observations and recording and reporting data in your laboratory manual. The Parts of the Investigations marked C involve individual experimentation. Since in these Parts you will be expected to report the findings of your studies, we have prepared an outline for you to follow. Your teacher may give you another outline to follow, or you may find one for yourself. In general, however, it should include the following elements:

I. *Title*—This should be specific. "Growing Plants" or "Plant Nutrition" are too vague as titles. The title should tell exactly what you are studying about plant nutrition. A good title might be: "The Effect of Mineral Deficiency on the Growth of Coleus Plants."

II. *Purpose*—This section should state the problem you are investigating. The statement should be simple. For example, how does the lack of certain minerals in the soil affect the growth of Coleus plants? Your entire report must relate to this problem.

III. *Materials and methods*—You must tell exactly what you did to prove or disprove the hypothesis that the lack of certain minerals affects the growth of Coleus plants. Be sure to have a control for your experiment. In this one, the control plant should be provided with a complete mineral supplement. All other conditions for the plants should be identical except for the mineral content of the soil.

Describe the exact experimental procedures and use of materials in the sequence in which you did them.

IV. *Observations and results*—This part of your paper is very important because it forms the basis of support for your analysis and conclusions. It is purely objective. *Do not attempt to interpret the data you collect* in this section. You must be sure that what you observe and measure are actually there. Do not overlook any result. Record *all* experimental data and observations you make. Give a complete description of what happened. Illustrations, drawings,

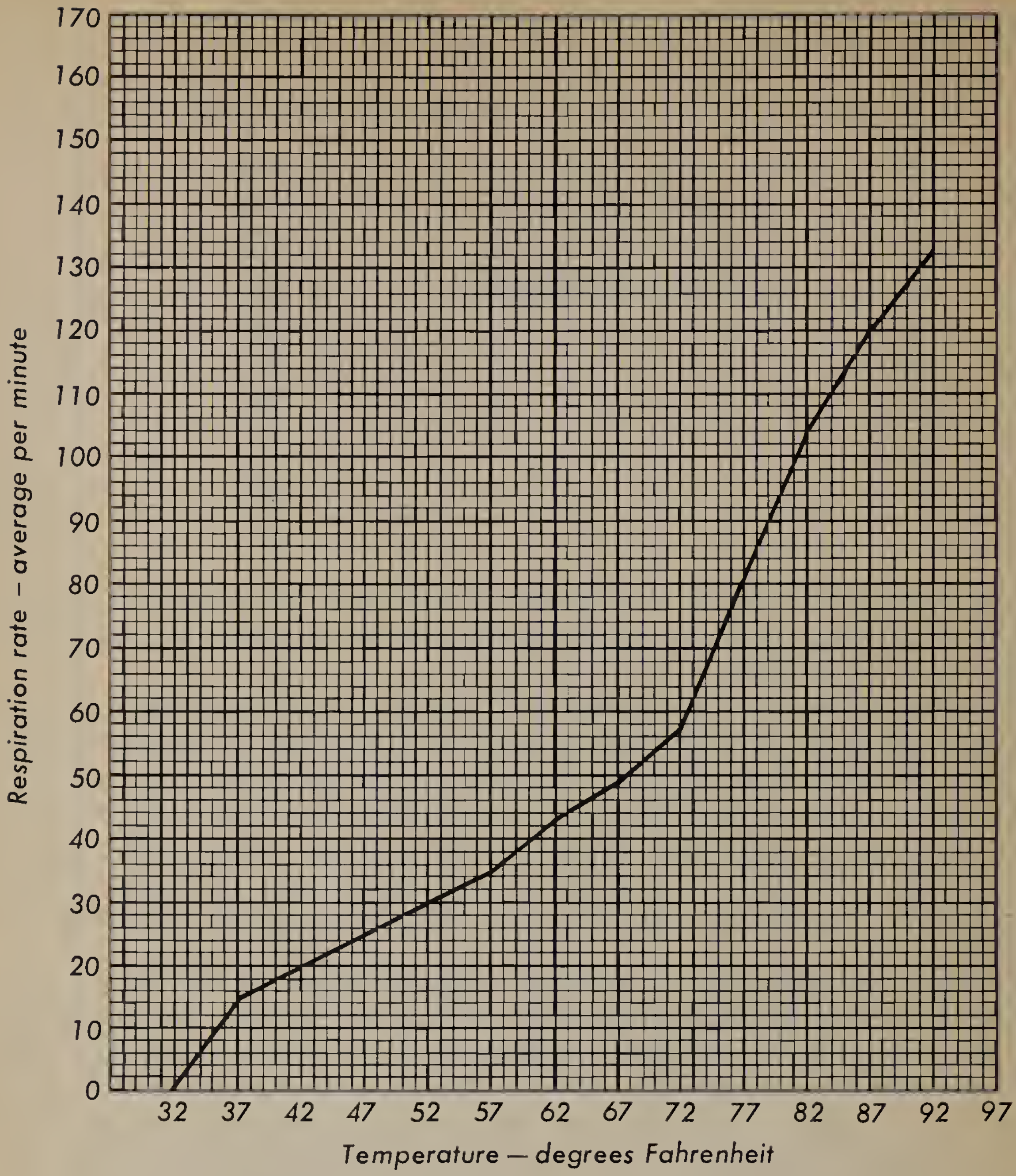


Fig. D The effect of temperature on the respiration rate in goldfish as indicated by operculum measurements

graphs, sketches, and data tables should be used to support the information whenever possible.

V. *Analysis and conclusions*—These are subjective in nature. Here you can interpret your results. Tell how your results prove or disprove your original hypothesis. Make each conclusion positive and distinct. You should leave no doubt in the mind of the reader as to why you feel justified in making your conclusions on the basis of the evidence you have collected.

Standard laboratory equipment. Although the names of standard pieces of biology equipment will be used throughout this manual, many simple substitutes may be used. An enlarging glass may be used in the place of a hand lens; manicure scissors do well for dissections; tweezers can be used in place of forceps; a single-edge razor can be used for cutting thin sections; a needle mounted in the end of a small stick does as well as any dissecting needle; a dissecting pan can be made by pouring a mixture of equal parts of melted paraffin and beeswax into a shallow, oblong cake pan; materials may be kept in shallow dishes and glass jars; mason jars, properly sealed, can be used to keep preserved materials. Students in the woodworking classes can probably be prevailed upon to make such items as test-tube racks, animal cages, aquariums, and terrariums.

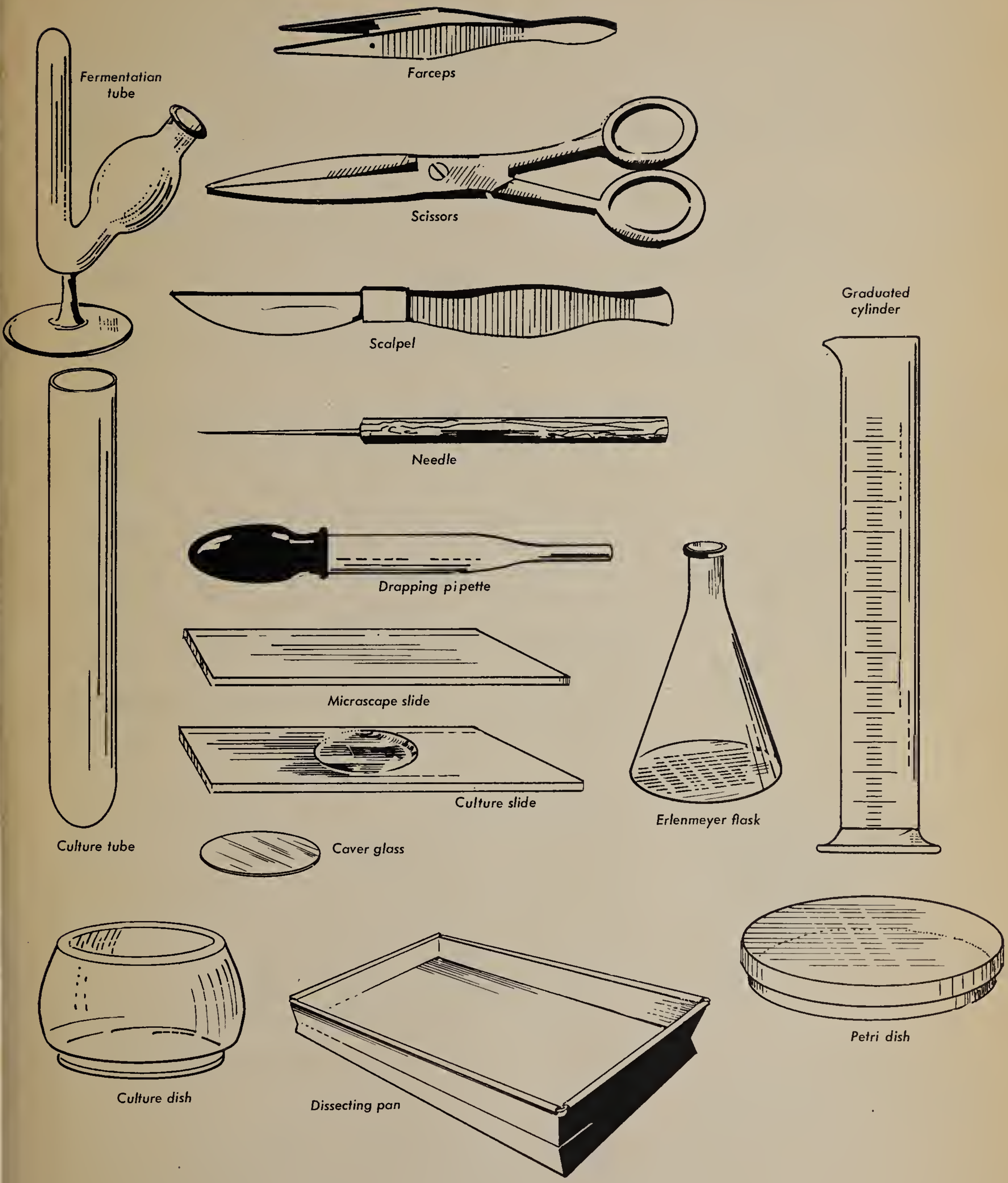

Fermentation tube
Forceps
Scissors
Scalpel
Graduated cylinder
Needle
Dropping pipette
Microscope slide
Culture slide
Erlenmeyer flask
Culture tube
Cover glass
Petri dish
Culture dish
Dissecting pan

Fig. E Pieces of apparatus frequently used in biological laboratories

Microscopes—Basic Tools of the Biologist 1-1

Purpose To become familiar with various types of microscopes and their uses and to learn the parts of the compound, or light, microscope.

There are several kinds of microscopes, each with a particular purpose. Some provide low magnifications for examining larger structures. The standard compound microscope you will use most frequently provides magnifications for studying cells and most microscopic organisms. Far beyond the range of this microscope is the new and remarkable electron microscope with magnifications of 100 to 1,000 times that of the standard microscope.

A Part 1 SIMPLE MICROSCOPES. A simple microscope has a single lens or a pair of lenses mounted close to each other, providing a direct, or one-stage, magnification. An ordinary magnifying or reading glass can be classified as a simple microscope.

Materials. Fresh leaves; hand lens; tripod magnifier; stand magnifier.

Procedure and observations. The hand lens shown in Fig. 1-1-a, top, is ideal for examining materials in the field. Various kinds of hand lenses fold into a protective cover or case. Lenses of this type are used for examining such subjects as small insects, structures on a twig, flower structures, details of a leaf, and other materials in the field. When viewing with a hand lens, the subject should be held 6-8 in. from the eye. The lens is held close to the eye and moved back and forth to obtain a clear focus. Hand lenses vary in magnification from 5-20 times. As the magnification increases, the diameter of the lens (field) decreases. (a) What is the magnification of your hand lens? ....
(b) What is the diameter of the lens in millimeters?

....

The tripod magnifier shown in Fig. 1-1-a, middle, is ideal for studying materials in the laboratory. It can be placed over a subject such as an earthworm or an insect and focused by screwing the lens up or down in the tripod stand. (c) What magnification is provided with your tripod magnifier? .... Place the tripod magnifier on the lower side of a fresh leaf and turn the lens until the focus is sharp. (d) What details can you see that were not clearly visible to the unaided eye? ....

....

Turn your back to the light and hold the leaf surface 6-8 in. from your eye. With the legs of the tripod magnifier turned out, hold the lens close to your eye. Move the leaf back and forth until it comes into sharp focus. You may wish to keep the leaf stationary and move the lens to bring it into sharp focus. (e) Why did you turn your back to the light? ....

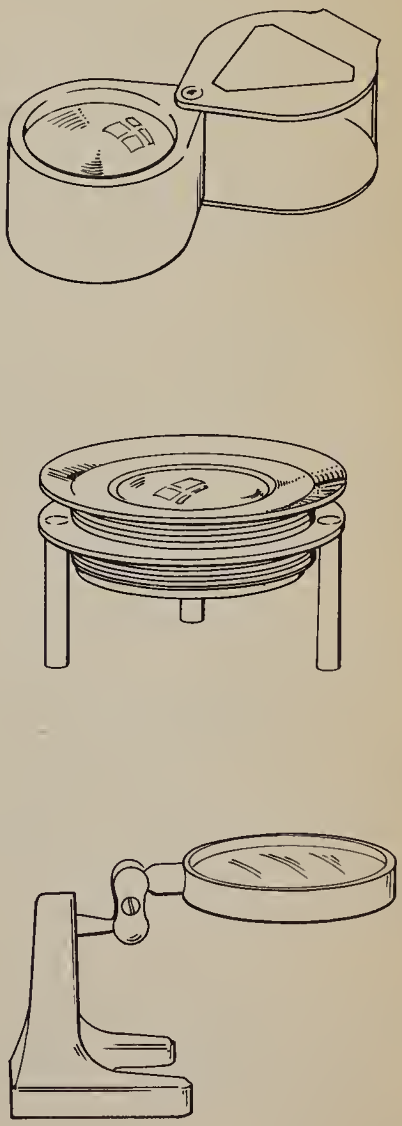


Fig. 1-1-a Simple microscopes

A stand magnifier, such as the one shown in Fig. 1-1-a, bottom, is designed to permit the use of both hands while working with a magnified subject. You will find this type of magnifier ideal in making a dissection of a small animal such as an earthworm, removing the mouthparts of a grasshopper, or removing the parts of a small flower. Stand magnifiers frequently magnify from 3-5 times and have a lens diameter of 50-100 mm. (f) What is the magnification of your stand magnifier? .... (g) What is the diameter of the lens?

....

A Part 2 THE STANDARD COMPOUND MICROSCOPE. The compound microscope has two separate lens systems, providing a two-stage magnification. Depending on the lenses in these systems, the compound microscope provides magnification from 50 to as much as 1,500 or 2,000 times. The most common magnifications are 100×, 430 or 440×, and 970 or 1,000×. This microscope is referred to as a light microscope because light passes through the material to be examined and through the lens systems to the observer's eye. Since light is transmitted through the material to be examined, subjects viewed must be very small or very thin. Some parts of the material will absorb more light than others, thus making it possible to distinguish form and structure in the magnified image that reaches the eye.

Materials. Standard compound microscope; metric ruler; drawing pencil.

Procedure and observations. A standard compound microscope is shown in Fig. 1-1-b. This microscope is equipped for use in high school and general use in college laboratories and is, therefore, referred to as a student microscope. The microscope shown has a single ocular, or eyepiece, and is, therefore, called a monocular microscope. Since it has a single ocular, you will view materials with only one eye. Therefore, you will see an image with length and width, but very little depth. It also has a vertical ocular. Some standard compound microscopes are equipped with an inclined ocular which is angled toward the observer.

The following is a list of the parts of a standard compound microscope. Locate and examine each part on your instrument. Label each part named in *italics* on the microscope shown in Fig. 1-1-b.

1. The *eyepiece*, or *ocular,* is the removable cylinder in the top of the microscope. It contains one set of lenses which magnify the number of times indicated on the top of the eyepiece as, for example, 5×, 7.5×, or 10×. (a) What magnification is marked on the top of the eyepiece of your microscope? ....

2. The *tube* is a hollow cylinder which forms the body of the microscope. The upper portion of the tube contains the eyepiece.

3. Fastened to the base of the tube is the circular *nosepiece.* The lower part of the nosepiece is movable and is called the *revolving nosepiece.*

4. The revolving nosepiece contains two or more small cylinders having magnifying lenses called *objectives.* (A microscope with a single objective has a stationary nosepiece.)

5. The *low-power objective,* the shorter of the two, contains a series of lenses which magnify an amount indicated on the side of the objective. A 10× objective is very widely used. The total enlargement, or magnification, is determined by multiplying the magnification of the eyepiece by the magnification of the objective. (b) What is the magnification of your microscope, using low power? ....

6. The *high-power objective,* the longer one, contains stronger lenses, giving greater magnification. A 43× objective is found on many microscopes. (c) Multiply the objective magnification by the eyepiece enlargement and determine the high-power magnification of your microscope. ....

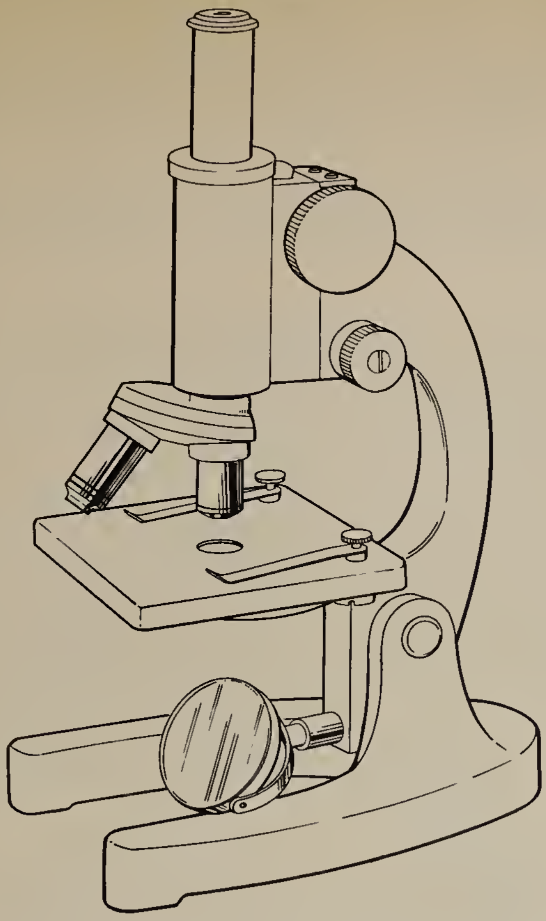


Fig. 1-1-b Standard compound microscope

7. The microscope is focused by turning one or two adjustments, depending on the type of instrument. The *coarse adjustment* consists of large knobs or wheels on the sides of the tube which, when turned, raise and lower the body tube.

8. The *fine adjustment* is used for sharp focus after the object has been brought into view with the coarse adjustment. Turning the fine adjustment knobs, below the coarse adjustment, raises and lowers the body tube, but so slightly that it can hardly be noticed. These knobs should usually not be turned more than a half-turn in either direction.

9. The *arm* is the curved part of the frame below the focusing adjustments. When you are carrying the microscope, you should grasp it firmly by the arm, supporting the bottom with your other hand, and carry it upright to avoid dropping the eyepiece.

10. The *stage* is a platform upon which material is placed for observation. An opening in the center of the stage permits light to pass from below through the object being studied.

11. A pair of *spring clips* mounted on the stage holds the material securely in place.

12. The *diaphragm* beneath the stage regulates the amount of light which passes through the lenses to the observer's eye. Some diaphragms are disks with holes of different sizes. Others have a series of sliding leaves which vary the size of the opening.

13. The *mirror* is fastened to the base of a movable arm and is used to direct light through the lenses. If the mirror has two faces, one flat and one concave, the concave face should be used under ordinary conditions.

14. The *base* is a heavy U-shaped anchor to hold the microscope in place.

15. The microscope frame is fastened to the base by a movable hinge, called the *inclination joint,* which allows the microscope to be tilted for a comfortable viewing position.

A Part 3 THE ADVANCED COMPOUND MICROSCOPE. The high power magnification of the standard compound microscope, 430 or 440×, is not sufficient for certain areas of biological study, including microbiology and many fields of medicine. The advanced compound microscope provides the same magnifications as the standard instrument and, in addition, is equipped to provide a magnification of 970 or 1,000 times. Special oculars may be used to increase this magnification to 1,500 or, in some, to 2,000 times. This is considered the upper limit of magnification with a light microscope.

Materials. Advanced compound microscope (if not available, the drawing in Fig. 1-1-c will suffice); ruler; drawing pencil.

Procedure and observations. The high magnification of the advanced compound microscope is provided by a special objective with a magnification of 97 or 100X. This special objective is usually mounted with the standard low-power and high-power objectives in a *triple nosepiece.* It is referred to as an *oil-immersion objective* because the tip of the objective is immersed in a film of oil on the microscope slide when the high magnification is used. This film of oil is necessary in providing a clear image with the oil-immersion objective. When used with a 10× ocular, the oil-immersion objective provides a magnification of 970 or 1,000 times.

Because the aperture (lens opening) of the oil-immersion objective is small, light must be focused to a fine beam at the objective tip. This is accomplished by a *condenser,* mounted on an adjustable substage below the microscope stage. The *diaphragm* below the condenser lens is used to regulate light. An artificial light source is often used to provide maximum illumination for the oil-immersion objective.

An advanced compound microscope is often equipped with a *mechanical stage.* This device holds the slide securely and has adjustments for moving the slide right or left and toward or away from the viewer. It is very useful in counting or measuring materials and in precision movement of the microscopic subject as it is being viewed.

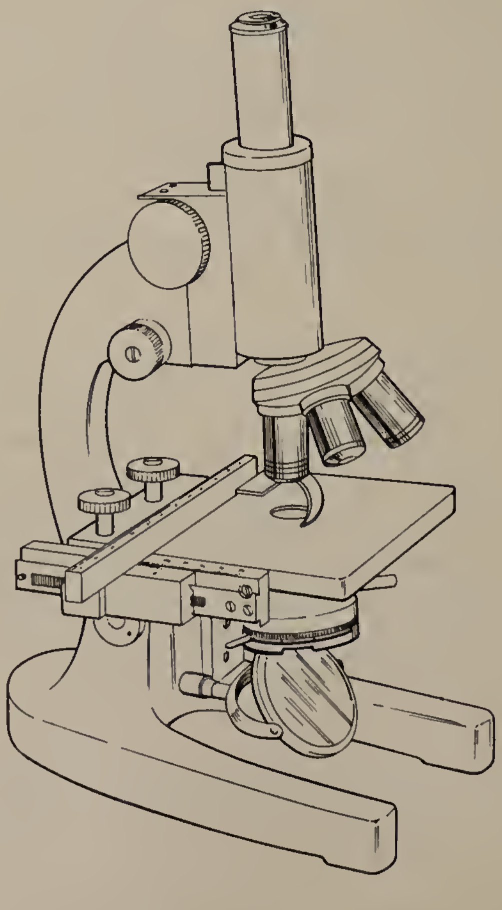


Fig. 1-1-c Advanced compound microscope

In the advanced compound microscope shown in Fig. 1-1-c, label: **triple nosepiece, oil-immersion objective** (the longest of the three), **mechanical stage, condenser, diaphragm.**

A Part 4 THE WIDEFIELD STEREOSCOPIC MICROSCOPE. Many materials with which a biologist works are too large for even the lowest magnification of a compound microscope. Furthermore, many are opaque and must be viewed with reflected light rather than transmitted light, as in the compound microscope. The widefield stereoscopic microscope is designed for this type of microscope work.

Materials. Insects, flower parts (or other small objects for viewing); widefield stereoscopic microscope.

Procedure and observations. A widefield stereoscopic microscope is shown in Fig. 1-1-d. Notice that it has paired oculars, one of which is focused independently of the other so as to provide a clear image for both eyes. The instrument also has paired objectives. Thus, it is actually two microscopes combined in one instrument.

The widefield stereoscopic microscope is a low magnification instrument. Its highest magnification is below the lowest magnification of the compound microscope. Various ocular and objective combinations provide magnifications ranging from 4× to as high as 30 or 40×. Magnifications are changed by changing oculars or paired objectives or both. This provides several different magnifications.

Notice that the distance between the stage and the objectives is much greater than that of the compound microscope. This provides a wide area for large objects and room to work while viewing a subject, as in making a dissection. Since magnifications are low, sufficient light for viewing reflects from the surface of the material on the stage.

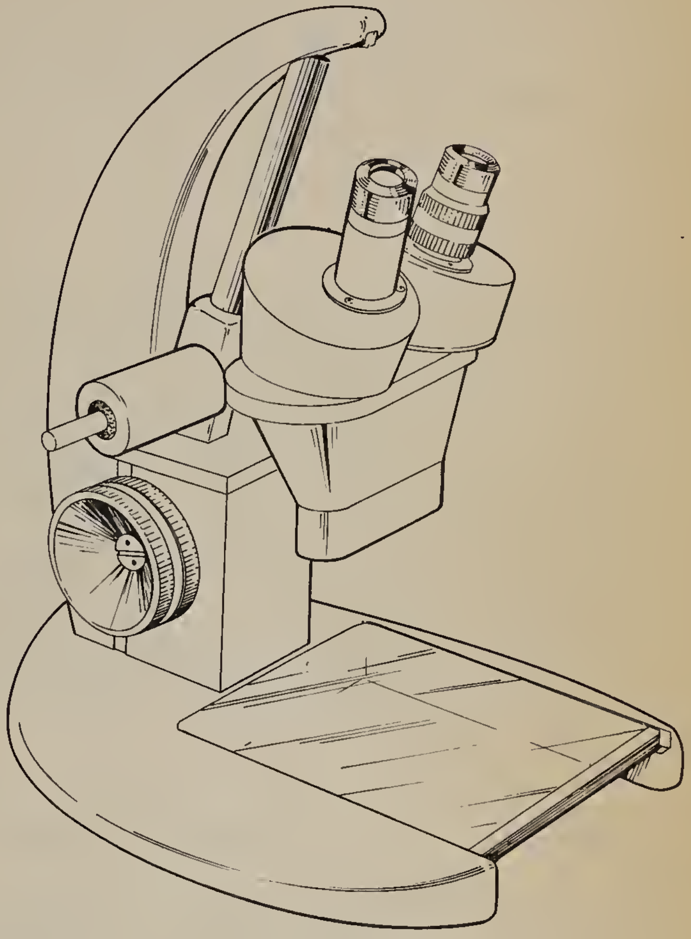


Fig. 1-1-d Widefield stereoscopic microscope

Put a small insect such as an ant or a fly, a small flower, or some other interesting subject on the stage of your microscope. Determine which eyepiece is focused independently. Adjust the position of the two oculars until you see a clear field with both eyes. Unless both oculars can be focused independently, look through the fixed ocular and bring the material into sharp focus by turning the focusing adjustment. Then, correct the focus for the other eye by adjusting the ocular. This should provide clear binocular vision and the material should stand out in three dimensions.

Move the material to the left. (a) Which direction does it move in the microscopic field?

.... Move the material away from you. (b) How is its position

changed in the microscopic field? ....

....

(c) What feature of the stereoscopic microscope produces a three-dimensional image?

(d) In what respect is this a distinct advantage in using the stereoscopic microscope?

If your microscope has more than one pair of oculars or objectives, change to the next higher magnification. (e) How much have you increased the size of the magnified image?

(f) Has a change occurred in the size of the field? Explain.

A Part 5 THE ELECTRON MICROSCOPE. The compound, or light, microscope reaches its upper limit of magnification at about 1,500×, although this magnification can be increased to as much as 2,000× with a special ocular. However, at these high magnifications, the resolving power is lost, and while materials are greatly enlarged, details do not appear in the magnified image. The properties of light itself limit the resolving power of the compound microscope. Generally, materials that are smaller than half the wavelength of the shortest waves of visible light (violet rays) cannot be seen with the light microscope.

The electron microscope solves this problem by substituting streams of electrons for light. These electron beams are of very short wavelength and high energy level. The lowest magnification of the electron microscope extends into the range of the highest magnification of the light microscope, while its greatest magnification is more than 100 times that of the light microscope.

Materials. No materials or apparatus required.

Procedure and observations. Figure 1-1-e shows a modern electron microscope and a diagram of its optical system. As the principle of the instrument is described, trace the path of the electrons from their source to the viewplate or photographic plate.

Electricity is used to heat the tungsten to a temperature of 2,000°C. This produces a cloud of electrons which boil off around the wire. An electron gun accelerates the electrons through the vacuum chamber of the microscope. Streams of electrons pass through two electromagnets known as the *condenser lens.* This lens shapes the beam so that it passes through the material to be magnified. The material to be examined is mounted on fine copper wire mesh. The electron beam passes through this material and enters two more sets of electromagnets, the *objective lens* and the *projector lens.* Both of these bend the electron beam and produce high magnification. The extremely short wavelengths of the electron beams are not visible. However, when they strike a phosphorus-coated plate enclosed in a leaded glass for safety, the plate glows and produces a visible image. This is the principle of the picture on a television picture tube. The operator uses the image on the viewplate to position the materials and focus the electron beams. He usually exposes a negative from which a photographic print can be made for study.

The amount of magnification of the electron microscope is determined by the electromagnets used. Magnifications range from about 1,400 to as much as 200,000 times. By enlarging the negative exposed at a magnification of 200,000 times five times, it is possible to produce a print 1,000,000 times the actual size of an object. However, no details will appear which the microscope did not "see."

The electron microscope has some limitations. Materials must be extremely thin to allow penetration of the electron beam. Since the specimen is to be examined in a vacuum tube, it must be dehydrated. For this reason, it has not been possible to study living cells under the electron microscope.

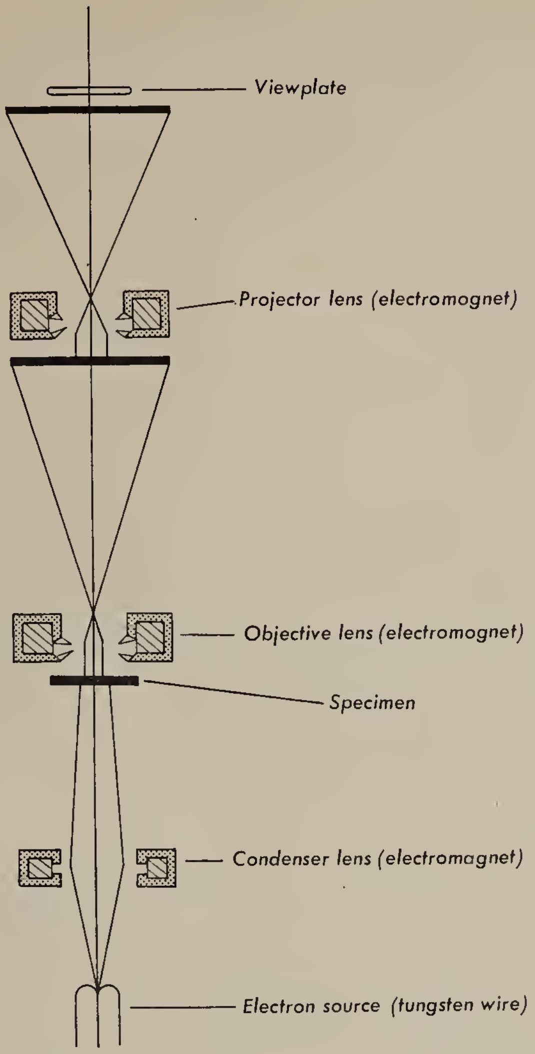

Viewplate
Projector lens (electromognet)
Objective lens (electromognet)
Specimen
Condenser lens (electromagnet)
Electron source (tungsten wire)

Fig. 1-1-e Diagram of optical system of electron microscope

for microscopic measurement. For measuring microscopic materials, the biologist uses a unit known as a micron (μ), which is 0.001 mm.

Research microscopes are often equipped with an *optical micrometer,* a glass disk with a micron scale which is placed in the ocular (Fig. 1-2-a). By moving the slide, an object may be placed along the scale and measured accurately. Without an ocular micrometer, however, you can determine the approximate size of many subjects by comparing them with the known diameter of the low-power and high-power fields.

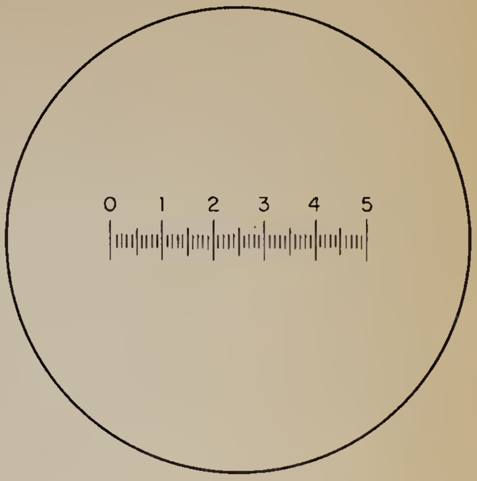


Fig. 1-2-a Optical micrometer

Materials. Microscope; plastic metric ruler; subject for microscopic measurement; slide; cover glass; dropping bottle; distilled water.

Procedure and observations. To measure the diameter of the low-power field, lay a plastic metric ruler across the stage opening. Find the ruler in the low-power field. Move the ruler so that one of the vertical lines dividing the ruler into millimeters is just visible at the left edge of the field. The distance between the first and second vertical lines is 1 mm. Since a third vertical line does not appear, you know that the diameter of the low-power field is less than 2 mm. It is necessary, therefore, to estimate the distance beween the second line and the right edge of the field. (a) What is the estimated diameter of the low-power field in millimeters? .... This measurement can be changed to microns (1 mm equals 1,000 μ). (b) Calculate the diameter of the low-power field in microns. ....

Since the diameter of the high-power field is less than 1 mm, it will be necessary to determine the diameter of this field by calculation in terms of the measured diameter of the low-power field. If your low-power objective magnifies 10 times and the high-power objective magnifies 43 or 44 times, dividing 43 or 44 by 10 gives a factor of 4.3 or 4.4. If you divide the diameter of the low-power field by this factor, you can determine the diameter of the high-power field. (c) What is the diameter of your high-power field in millimeters? ....

(d) in microns? ....

Select a subject you can measure in length and width in your microscopic field and prepare a wet-mount slide. Move the subject to the left edge of the field and estimate its length, then its width. (e) What subject did you use? ....

(f) What is its estimated length in millimeters? .... (g) in microns? ....

(h) What is its estimated width in millimeters? .... (i) in microns? ....

Growth Characteristics of Living Organisms

Purpose To establish experimental evidence of biogenesis as opposed to the theory of spontaneous generation; to determine whether plants grow by adding soil substance.

Redi, Spallanzani, Pasteur, and van Helmont made important contributions to science when they provided scientific evidence to disprove the theory of spontaneous generation. False ideas, myths, and superstitions about the origin and growth of living organisms were deeply rooted in the thinking of people in earlier times. The fact that the experiments of Redi and Pasteur were separated by three centuries is indication of the difficulty early investigators faced in replacing superstitions with demonstrated truths. In this Investigation, you will repeat certain experiments of earlier scientists who laid important groundwork for modern biological understandings and concepts.

B Part 1 DEMONSTRATION OF BIOGENESIS OF MICROORGANISMS. Long after Redi proved that decaying meat could not produce flies by spontaneous generation, many still believed that various nonliving substances could change to bacteria and other microorganisms. Spallanzani and Pasteur supplied the scientific proof that finally refuted the theory of spontaneous generation in experiments similar to the one you will conduct in this Part.

Materials. Microscope; slides; cover glasses; six 250-ml flasks; 2 solid stoppers and 2 one-hole stoppers to fit the flasks; 3-in. section of straight glass tubing; S-shaped glass tube; 6 dropping pipettes; pan for preparing broth; autoclave or pressure cooker; glass-marking pencil (heat resistant); aluminum foil; nutrient broth (dehydrated) or beef broth; distilled water.

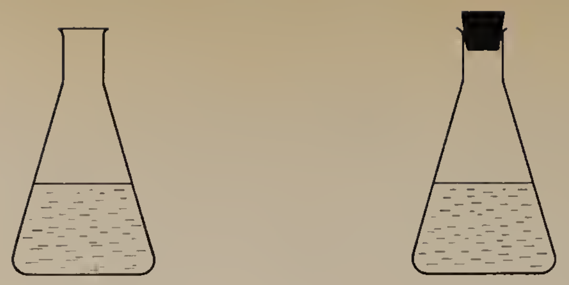
Procedure and observations. Insert a section of straight glass tubing and an S-shaped piece of glass tubing through one-hole stoppers as shown in Fig. 2-1-a, flasks E and F. Your teacher will give you specific directions for inserting glass tubing in rubber stoppers.

Prepare 1,000 ml (1 liter) of nutrient broth, using distilled water as directed on the media bottle. Stir until all ingredients of the broth are dissolved. Identify six 250-ml flasks as *A* through *F* with a heat-resistant glass-marking pencil. Divide the broth equally in the six flasks. Prepare all flasks as follows and as shown in Fig. 2-1-a.

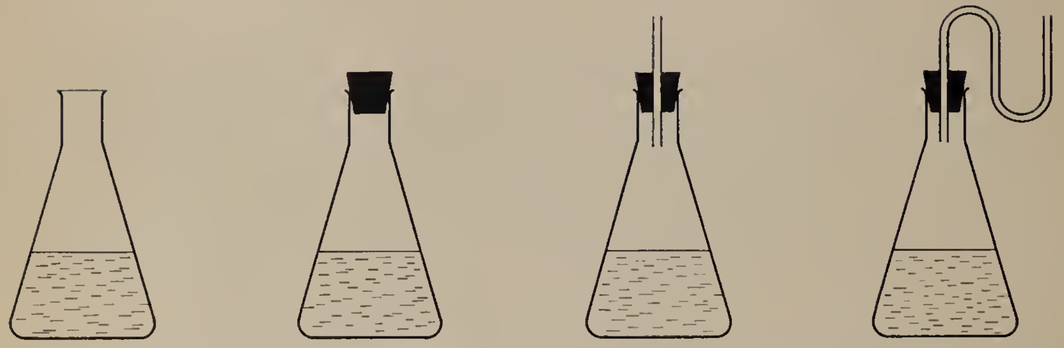
Flask A will remain unsterilized and will be left open
Flask B will remain unsterilized and will be stoppered
Flask C will remain open after sterilization
Flask D will be stoppered after sterilization
Flask E will be stoppered with the straight glass tube after sterilization
Flask F will be stoppered with the S-shaped glass tube after sterilization

Prepare flasks A and B and set aside. Cover the mouths of flasks C, D, E, and F with aluminum foil and put these flasks and the stoppers for flasks D, E, and F in the autoclave or pressure cooker. Sterilize at 15 pounds for 15 minutes. After sterilization, allow the flasks and stoppers to cool in the sterilizer. Remove the aluminum foil from the four flasks. Insert the sterilized stoppers in their respective flasks, using care not to touch the lower part of the stoppers. Put all six flasks in the incubator. Incubate at 35-37°C for 72 hours.

At the end of the incubation period, examine all flasks. Using a clean dropping pipette for each flask, put a drop of broth on a microscope slide, add a cover glass, and examine with high power. If bacteria or other microorganisms are present, they will appear as very small moving or quivering bodies. It may be necessary to darken your field to see these microorganisms. In the table of observations, indicate the presence or absence of microorganisms in the broth in each flask. Examine the broth in each flask for evidence of physical changes such as



A *Unsterilized, open* B *Unsterilized, stoppered*



C *Sterilized, open* D *Sterilized, stoppered* E *Sterilized, straight tube* F *Sterilized, S-shaped tube*

Fig. 2-1-a Method of preparing flasks A-F

color change, degree of turbidity (cloudiness), odor, surface pellicle (film), and sediment. These changes indicate activity of microorganisms in the broth. Record your observations for each flask in the table.

Flask	*A*	*B*	*C*	*D*	*E*	*F*
Presence of microorganisms						
Color						
Degree of turbidity (cloudiness)						
Odor						
Surface pellicle (film)						
Sediment						

Interpretation. Supporters of the theory of spontaneous generation, in examining the results shown in your table, would have maintained that substances in the broth changed to microorganisms in those flasks in which you found living organisms. They might have argued, also, that the heat used in sterilization of four of the flasks destroyed an "active principle" necessary for spontaneous generation and that closing two of the flasks with solid stoppers interferred with spontaneous generation by preventing the entry of air.

Spallanzani and Pasteur would have viewed the results differently. They would have maintained that microorganisms already present in the unheated broth multiplied during incubation or that they entered from the air.

Could spontaneous generation have occurred? Was an "active principle" destroyed by heating the broth? What was the source of microorganisms in certain of the flasks? An evaluation of your results in each flask should provide these answers. Use the following key in evaluating each of the theories or assumptions relating to your table of results:

+ evidence to prove
? possibility, but evidence for proof lacking
– evidence to disprove
0 does not apply.

Flasks	*A*	*B*	*C*	*D*	*E*	*F*
Living organisms were present in the broth before incubation						
Broth substances were changed to living organisms by spontaneous generation						
Heat destroyed an "active principle" in broth necessary for spontaneous generation						
Air is necessary for the growth of organisms in broth						
Organisms present in the broth came from the air						
Organisms will not appear if neither the broth nor the air entering the flask contain organisms						

Using your evaluation of results of experimental evidence shown in various flasks you used, construct a proof that living organisms cannot be formed from nonliving materials in broth by spontaneous generation. ....

....

....

....

....

....

C Part 2 PLANT GROWTH AND SOIL SUBSTANCES

Problem. Does a plant grow by changing soil to plant substances?

Another early theory, related to the theory of spontaneous generation, was that plants grew by converting soil to plant substances. If this were the case, each plant would use an amount of soil equal to its own weight. Early in the 17th century, J. B. van Helmont disputed this theory and conducted his famous experiment with a willow tree. He planted a five-pound tree in a tub containing 200 pounds of soil. At the end of five years, he removed the tree and weighed it. It now weighed 169 pounds—a gain of 164 pounds. How much of this substance had come from the soil? The tub still contained 199 pounds and 14 ounces of soil. Two ounces of soil lost—164 pounds of tree gained; van Helmont proved his point!

You can perform a similar experiment, reducing five years to three or four weeks and substituting a sunflower for a willow tree.

Suggested materials. Sunflower seedling; oven; 4 or 5 in. flowerpot; potting soil (sufficient to fill flowerpot); pan for drying soil; platform balance.

Suggested procedure. Previous to the experiment, grow several sunflower seedlings to a height of three inches in a flat or small flowerpots.

Fill a 4 or 5 in. flowerpot with potting soil. Empty the soil into a large, shallow pan and spread it evenly. Dry the soil in an oven at 250-300°F for one to two hours. Weigh the flowerpot, then fill it with dry soil. Weigh the flowerpot and soil and determine the weight of the soil.

Select a vigorous sunflower seedling and remove it from the flat or small flowerpot. Wash all of the soil from the roots. Remove excess water, then weigh the seedling. Record the weights of the soil and seedling at the start of the experiment. Plant the sunflower in the dried soil and water it well. Place the plant in a well-lighted area of the room and water as needed. Grow the plant three or four weeks.

At the end of the experiment, remove the plant from the soil, using care not to destroy any of the roots or lose any of the soil. Weigh the plant. Dry all of the soil in the oven, then weigh it.

Analysis and conclusions. Summarize your data in a table or as a line graph showing gain in weight by the plant and loss of soil during the growing period. In drawing conclusions from your data, consider all possible sources of substances used by the growing plant. Van Helmont thought the gain in weight of the plant represented intake of water entirely. Did the plant use substances from other sources? Account for as many possibilities as you can in presenting your conclusions. These will, of course, be theories since they were not proved in your experiment.

Purpose To investigate the properties of solutions, colloids, and suspensions.

The relation of a solid to the dissolving or dispersing liquid in a solid-liquid system is of great importance in biology. In what form do chemical substances enter the cells of living organisms? How are they transported? How are they involved in the many substances that compose living matter?

The size of dispersed particles determines whether a solid-liquid system is a solution, a colloid, or a suspension. We shall investigate various physical properties of these three systems.

A Part 1 PROPERTIES OF SOLUTIONS, COLLOIDS, AND SUSPENSIONS. Do the solid particles remain dispersed through the liquid indefinitely in all three of these solid-liquid systems? Can the solid particles be detected with a beam of light? Can they pass through a membrane? Can they be filtered? These are characteristics of solutions, colloids, and suspensions that are important to a biologist.

Materials. Gelatin; fine sand; six 600-ml beakers; three 1,000-ml beakers; four 6-in. culture tubes (test tubes); 3 sheets, white paper; light source (illuminator, slide projector, or flashlight); cardboard, 2 in. square; ring stand; clamp; string; three 6-in. lengths dialysis tubing; 3 funnels, glass; funnel rack; filter paper; tripod; Bunsen burner; distilled water; sodium chloride; nitric acid, conc.; silver nitrate sol'n.

Procedure and observations. Section 1 *Preparation of test solid-liquid systems.* A solution, a colloid, and a suspension will be used in the tests included in this Part. To prepare these, identify three 600-ml beakers as *1*, *2*, and *3*. Prepare the solution by adding 15 gm of sodium chloride to 500 ml of distilled water in beaker 1. Add the salt to the water slowly. Stir thoroughly and be sure that none of the salt settles to the bottom.

To prepare the colloid, add 3 gm of gelatin to 200 ml of hot distilled water (just below boiling) in beaker 2. Add the gelatin slowly and stir constantly.

Prepare a suspension by adding 3 gm of fine sand to 200 ml of distilled water in beaker 3. Stir the water and sand together.

Section 2 *Testing for settling effect.* Place the three beakers on sheets of white paper. This will enable you to see any solid particles that settle to the bottom. Stir the three mixtures thoroughly, then allow them to stand five minutes. Examine for settling out of the solid substances. If settling occurs, mark a + in the appropriate column of the table provided. If no settling occurs, indicate the result as −. (a) In which beaker or beakers did settling occur?

.... (b) What factor determines whether solid particles remain in solution or suspension or settle out? ....

Section 3 *Appearance of test materials in a beam of light.* Light rays penetrate solutions readily because of the extremely small size of the dispersed ions and molecules. However, the large particles in a colloid interrupt light rays and refract them. This causes a milky appearance. The large particles in suspensions also interrupt light rays, but soon settle out and lose this effect. To determine this light effect, use a light source (an illuminator or slide projector may be used). Punch a small hole in the center of a piece of cardboard 2 in. square and hold it in front of the lens. This should produce a narrow beam of light. Stir each of the test liquids, then hold the beaker in the path of the beam of light. In the case of the sand, notice the change in appearance as settling occurs. Record your observations in the table provided, indicating penetration of the test liquid as + and refraction of light from the solid particles as −.

Section 4 *Testing for filtration effect.* The size of the solid particles in solutions, colloids, and suspensions also determines whether the system can be filtered without being altered. To determine this characteristic of each of your test materials, place three glass funnels in a funnel rack. Put a 600-ml beaker under each funnel. Line each funnel with a piece of standard filter paper (fold as demonstrated by your teacher). Pour approximately 50 ml of each test liquid into a funnel and allow it to filter until all of the liquid has passed through. Note any difference in the rate of filtration.

You can determine whether sodium chloride particles (sodium ions and chloride ions) pass through the filter paper by testing with silver nitrate solution. Pour 2 ml of the filtrate into a culture tube (test tube) and add 2 or 3 ml of silver nitrate solution. A white precipitate indicates the presence of chloride ions.

Gelatin is a protein and can be tested with concentrated nitric acid as follows. Pour 2 ml of the filtrate from the second beaker into a culture tube. Slowly, add about 2 ml of concentrated nitric acid. (CAUTION: *Use extreme care in adding the acid.*) Protein turns yellow in the presence of nitric acid.

If any of the sand passes through the filter paper, you can detect it in the bottom of the beaker in which you received the filtrate.

Indicate your results in the table, using a + if particles of the solid pass through the filter paper and a − if they do not pass through. (c) Did all of the test liquids filter at the same rate? .... (d) If not, list them in the order in which filtration was completed.

....

....

Section 5 *Passage of test substances through a membrane.* Fill three 1,000-ml beakers to within about two inches of the rim with distilled water. Then cut three 6-in. lengths of cellulose dialysis tubing. Submerge each in a beaker of water and allow it to soak half an hour, or until it is soft and pliable. Pour 50 ml of each test liquid into a dialysis tube. Tie both ends of the tubes securely with string, then tie the ends together to form a loop. Put the beakers of distilled water on ring stands to which clamps are attached. Suspend each dialysis tube containing a test liquid from the clamp as shown in Fig. 3-1-a. Be sure that the level of the test liquid in each dialysis tube is below the level of the water. Allow the tubes to remain immersed 15 to 20 minutes (the time should be the same for all three test liquids).

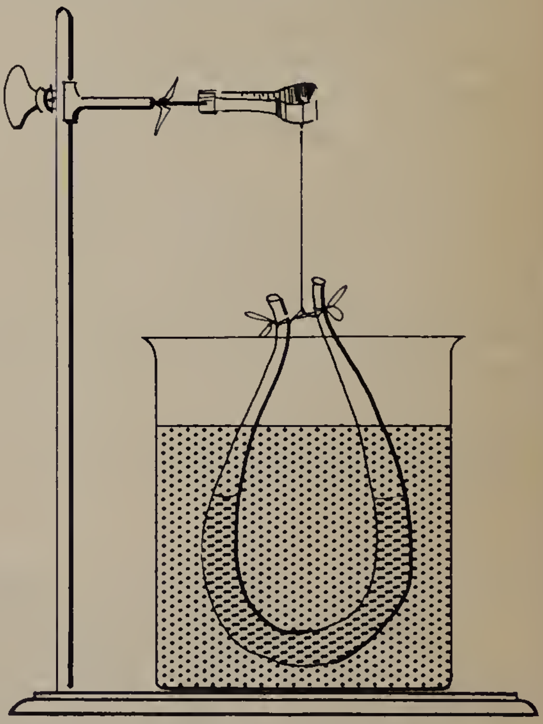


Fig. 3-1-a Setup with cellulose dialysis membrane

To determine whether chloride ions passed through the dialysis tubing, remove 2 ml of distilled water from the first beaker and test with silver nitrate solution as in Section 4.

The presence of gelatin particles in distilled water in the second beaker can be determined by means of the nitric acid test described in Section 4.

Any sand particles that passed through the dialysis tubing in the third beaker will appear as sediment at the bottom.

Indicate the passage of solid particles through the dialysis tubing as + and failure to pass as − in the table provided.

Section 6 *Determining the property of the test liquids to form a gel.* Many colloids have the property of changing from a liquid, or *sol,* state to a semisolid, or *gel,* state with temperature changes. To determine whether any of the test liquids will form a gel if the temperature is lowered, put the three beakers in a refrigerator (40-45°F) for 30 minutes to one hour. Indicate the property of forming a gel as + in the table and lack of this property as −.

Test liquid	*Type of mixture*	*Settling effect*	*Light penetration*	*Passage through a filter*	*Passage through a membrane*	*Property of forming a gel*
Sodium chloride and water						
Gelatin and water						
Sand and water						

B Part 2 DETERMINING THE PROPERTIES OF A CLAY SUSPENSION. Various kinds of clay differ in the size of the particles composing them. In some of the coarser clays, the particles are sufficiently large to form a suspension in water. However, the particles of fine clays are extremely small and form a colloid or a suspension bordering on a colloid in a water mixture.

Materials. Fine clay (you may wish to test several kinds of clay); materials listed for the tests in Part 1.

Procedure and observations. Add 3 gms of fine clay to 200 ml of distilled water in a 600-ml beaker. Stir the mixture until the clay is thoroughly dispersed in the water. Repeat all of the tests outlined in Part 1 and indicate your results in the table provided. (You may wish to extend your investigation to include a variety of clays. If several kinds of clay are used, be sure to indicate the source and physical appearance of each.)

Kind of clay	*Type of mixture*	*Settling effect*	*Light penetration*	*Passage through a filter*	*Passage through a membrane*	*Property of forming a gel*

Interpretation. Compare the properties of the clay and water mixture with those of the known colloid (gelatin and water) and the known suspension (fine sand and water) used in Part 1. On this basis, indicate the kind of solid-liquid system formed by the clay and water. ....

....

....

....

....

....

B Part 3 **STUDY OF REVERSIBLE AND IRREVERSIBLE COLLOIDS.** External conditions such as temperature changes may cause phase reversals in colloids. Some colloids are reversible, since they change from a liquid, or sol phase, to a gel and back to a sol with variations in temperature. Others are irreversible, since they do not return to a sol state after changing to a gel or semisolid.

Materials. Culture tube one-half full of gelled agar; egg albumen; 600-ml beaker; culture tube; tripod; test-tube holder; Bunsen burner; centigrade thermometer −10 to 110°C.

Procedure and observations. Fill a 600-ml beaker two-thirds full of tap water at room temperature. Put a culture tube one-half full of gelled agar in the water and put the beaker on a tripod. Put a thermometer in the water and heat slowly with a Bunsen burner. Watch closely for the first evidence of solation (liquefaction) of the agar along the side of the culture tube. It may be necessary to lift the tube out of the water with a test-tube holder and tilt it to determine when solation begins. (a) Record the solation temperature of agar. .... Continue heating the tube until all of the agar has liquified. This will require several more minutes.

When all of the agar is liquified, remove the beaker from the tripod and allow the water to cool. You may speed up the cooling process by putting the beaker in a pan of cool water. However, when the water temperature reaches 45°C, the beaker should be removed to allow the water to cool more gradually. Watch for the first evidence of gelation of the agar along the side of the tube. (b) Record this temperature as the gelation temperature of agar. ....

(c) Explain why agar is classed as a reversible colloid. ....

....

....

(d) List one or more other familiar colloids that undergo similar phase reversals with heating and cooling. ....

....

To illustrate a phase change in another colloid, fill a culture tube half-full of egg albumen. Put the tube in a 600-ml beaker of tap water at room temperature and repeat the procedure used with the agar. (e) Record the exact temperature at which the albumen starts to coagulate (solidify) along the side of the tube. .... Continue heating until all of the albumen has coagulated. Remove the beaker and cool as before. Watch for evidence of the coagulated albumen changing to a sol phase. (f) Does this occur? .... (g) If so, at what temperature? .... (h) Is egg albumen a reversible or an irreversible colloid? ....

Interpretation. On the basis of the phase changes you have demonstrated with colloids, compare these properties of colloids with physical changes in substances such as water as they relate to temperature variations. ....

....

....

....

Study of Organic Compounds Synthesized by Organisms

3-2

Purpose To test for the presence of carbon in a carbohydrate; to determine the properties of various organic compounds synthesized by organisms; to observe the behavior of organic nutrients in water; and to determine which of these organic nutrients will diffuse through a membrane.

During your course in biology, you will have many occasions to identify and work with organic substances synthesized by organisms. You will explore photosynthesis, a process by which green plants produce complex molecules such as sugars and starches. During further synthetic processes, organisms construct complex molecules of fats and proteins. In your study of digestion, you will become familiar with the breakdown of the organic compounds into simpler ones, their absorption into cells, and the reorganization of these simple substances into complex ones.

A Part I TEST FOR THE PRESENCE OF CARBON IN A CARBOHYDRATE. The sugar you use on your breakfast cereal or to sweeten your lemonade is composed of organic molecules. It was synthesized from simple sugars by cells of the sugar cane or sugar beet. Sugars and starches are carbohydrates. Potatoes and beans contain starch. Have you ever noticed that when potatoes are burned, they turn black? This is a chemical property of carbon. In this Part you will test sugar crystals to determine whether carbon is present.

Materials. Crucible; tripod; Bunsen burner; culture tube (test tube); test-tube holder; test-tube rack; sugar crystals.

Procedure and observations. Put a small amount of sugar crystals or a cube of sugar in a crucible. Put it on a tripod and heat with a Bunsen burner. Watch the sugar carefully as it is heated. (a) What happens to the sugar crystals? ....

.... Using a test-tube holder, hold an inverted culture tube above the crucible to catch the material being given off. Remove the tube and let it cool in an upright position. Examine the tube. (b) What do you find? ....

.... (c) What other substance was probably present in the vapor? .... Continue to heat the sugar until a black residue has formed in the bottom of the crucible. (d) What is this residue? ....

.... (e) What happened to the hydrogen and oxygen atoms that were present in the sugar molecules? ....

....

(f) What kind of change occurred in the sugar? ....

(g) Explain your answer. ....

.... (h) Was there an energy change? ....

(i) Explain your answer. ....

....

A Part 2 TEST FOR THE PRESENCE OF ORGANIC NUTRIENTS. You can determine whether sugar, starch, fat, or protein molecules are present in organic substances by using certain chemical tests.

Materials. Culture tubes (test tubes); test-tube holder; test-tube rack; Bunsen burner; brown paper; molasses or corn syrup; sugar; corn starch; fat or oil; hard-boiled egg white; Benedict's solution or Fehling's solution A and B; iodine; Sudan III; nitric acid; ammonium hydroxide.

Procedure and observations. Section 1 *Test for simple sugar.* The formation of a brick red color with Fehling's solution is a positive test for simple sugar. Put a few drops of corn syrup or molasses in a culture tube and add Fehling's solution A and B, filling the tube one-third to one-half full. (a) What color appears? .... Now heat the tube gently, being careful not to point the open end of the tube toward yourself or anyone else. Watch carefully for color changes during heating. (b) Record all color changes. ....

(c) What color finally appears? .... (d) Is the test for simple sugar positive or negative? ....

Now repeat the process, using cane sugar instead of corn syrup. (e) What color appears after heating? .... (f) Is the test for simple sugar positive or negative? ....

Section 2 *Test for starch.* Iodine turns blue-black in the presence of starch. Put a small pinch of corn starch in a culture tube and fill the tube about one-third full with water. Heat gently until the water boils. Add a few drops of iodine solution. (g) What color appears?

.... (h) Is the test for starch positive or negative? ....

Section 3 *Test for fats and oils.* Brown paper and Sudan III are separate tests for the presence of fats and oils. The brown paper becomes translucent when fat or oil is rubbed on it. Sudan III is oil soluble and will diffuse into oil droplets, turning them pink.

Spread a small amount of butter (lard) or put a drop of oil on a piece of brown wrapping paper. Hold the paper up to the light. (i) Can you detect a grease spot on the paper?

.... Put about one-fourth inch of butter or oil into a *dry* culture tube and add 10 drops of Sudan III. Set the tube aside for 30 minutes. (j) Examine the contents of the culture tube and discuss the results. ....

....

....

(k) Are the results for the test for presence of fat positive or negative? ....

Section 4 *Test for proteins.* Nitric acid is used to test for the presence of a protein. A yellow color indicates the presence of protein. Addition of a base, ammonium hydroxide, will cause the protein to turn orange.

Put a piece of hard-boiled egg white in a culture tube and cover with concentrated nitric acid. (CAUTION: *Use care in handling nitric acid!*) Warm the acid *gently* for a minute or two, but *do not boil.* Carefully pour off the nitric acid and rinse the egg with water, using care not to pour the egg from the tube in removing the acid and rinse water. (l) What color is the treated egg? .... Now add ammonium hydroxide solution, allowing it to run down the side of the tube. (m) What color does the egg turn after treatment with ammonium hydroxide? .... (n) Was the test for protein positive or negative? ....

B Part 3 IDENTIFICATION OF UNKNOWN ORGANIC NUTRIENTS. The foods you eat are composed of carbohydrates, fats, and proteins, which are necessary for the maintenance of cellular activities in both plants and animals. These nutrients are often combined in foods so that you cannot recognize them. The chemical tests you learned in Part 2, however, may be used to identify the composition of foods.

Materials. Various foods such as lean beef, raisins, dried beans, peanuts, apple, onion, popcorn, banana, skim milk, and whole milk; culture tubes (test tubes); test-tube holder; test-tube rack; Bunsen burner; Benedict's solution or Fehling's solution A and B; iodine; Sudan III; brown paper; nitric acid; ammonium hydroxide.

Procedure and observations. You are to test the food you are given for the presence of simple sugar, starch, fats and oils, and protein. Record a plus (+) if positive and a minus (−) if negative.

Food Tested	*Organic Nutrients*			
	Sugar	*Starch*	*Fats and oils*	*Protein*
Lean beef				
Raisins				
Dried beans				
Peanuts				
Apple				
Onion				
Popcorn				
Banana				
Skim milk				
Whole milk				

Interpretation. Account for the fact that many of the foods you tested contained one or more organic nutrients. ....

....

....

....

Study of Cells

4-1

Purpose To examine cells and to become familiar with the specialization of protoplasm.

The cell is the basic unit of life, and the individual part of which the whole organism is composed. Many of the substances in a cell are organized into specific organelles. You will be able to study some of these organelles with your light microscope.

A Part 1 STUDY OF CORK CELLS. When Robert Hooke first observed cells, he saw only the cell walls of the cells that form cork tissue. You will begin your observation of the cell by studying cork cells in the same manner as Hooke first observed them.

Materials. Bottle cork; microscope; slides; cover glasses; razor blade.

Cork cells (L.P.)

Procedure and observations. Using a razor blade, shave a thin section from an ordinary bottle cork. Mount it on a slide in a drop of water and add a cover glass. Examine the specimen under the low power, studying all portions by moving the slide. Locate the thinnest edges in order to have a clearer view of the cork cells. Draw a group of cells in the space provided. (a) Are the cells similar in shape? .... (b) Are they similar in size? ....

(c) Do the cells appear to have content? .... (d) Explain why or why not. ....

.... (e) Are there spaces between the cells? ....

A Part 2 STUDY OF ONION EPIDERMAL CELLS. The epidermis of the onion is an ideal subject for cell study because it is composed of a single layer of cells, and you can distinguish the individual organelles. As you study these cells, you are looking into functioning units of living material.

Materials. Onion; microscope; slides; cover glasses; forceps; scalpel; filter paper; iodine stain.

Procedure and observations. Cut an onion lengthwise. Remove a thick scale and peel the transparent tissue from the inner surface. Cut a square (¼ in.) section of the tissue and mount it on a slide in a drop of water. (CAUTION: *Avoid wrinkling the tissue.*) Add a cover glass. Examine the living cells under low power and high power. (a) What is the shape of the cells? ....

.... (b) Are they similar in form? .... (c) What color is the living protoplasm? .... (d) Do you find any movement? ....

Raise one side of the cover glass and add a drop of iodine stain. (e) What effect does iodine have on the cells? .... (f) What is the advantage of using the iodine stain? ....

(g) What is the appearance of the cytoplasm? ....

(h) What is the appearance of the nuclei? ....

(i) Are the nuclei always in the same position in the cell? ....

(j) Does the onion epidermial cell have depth? .... (k) Explain your answer. ....

.... (l) What is the function of the vacuole? .... Study the cells very carefully under low power and note that each cell has a cell wall. In the space provided, draw a small group of cells, making each cell about an inch long. Show the cell wall and nucleus of each cell and label: **cell wall** and **nucleus.**

Select one cell that shows the contents clearly. Move it to the center of the microscopic field. Using high power, examine all the parts of the cell. As you study the cell, shift the focus with the fine adjustment and note that the cell has thickness as well as length and width. You should see the cytoplasm filling most of the cell and its outer edge, the plasma membrane. The cavities in the cytoplasm are vacuoles, each bounded by a vacuolar membrane of the cytoplasm. The droplets in the cytoplasm are oil droplets. Draw in the space provided a single cell from the high-power field, making it about two inches long and including the nucleus. Label: **plasma membrane, cytoplasm, vacuolar membrane, vacuole, oil droplet, nucleus.**

Group of onion epidermal cells (L.P.)

In the space to the right of this cell, show the nucleus enlarged to about the size of a quarter. Show the nuclear membrane, dense nucleoplasm, and nucleolus (there may be several nucleoli). Label: **nuclear membrane, nucleoplasm, nucleolus.**

Onion epidermal cell (H.P.)

Nucleus (H.P.)

A Part 3 **OBSERVING MITOCHONDRIA.** It is possible to see the mitochondria of some cells with the light microscope.

Materials. Celery (fresh); microscope; slides; cover glasses; razor blade or scalpel; filter paper; 5% sucrose solution; 0.001% Janus Green B solution.

Procedure and observations. Cut a strip one centimeter long containing two of the "strings" from a stalk of celery. Place this strip, inner surface up, in a 5% sucrose solution. Using a razor blade or sharp scalpel, cut between the strings and parallel to them. You will have a thin strip, two or three cells thick, of the epidermis and underlying tissues. Put a cover glass over this thin strip. Observe under low and high power. (a) Can you see the streaming cytoplasm? .... (b) What cell organelles can you locate? .... The rods and spheres you see moving in the cytoplasm are the mitochondria.

Place a strip of filter paper at the edge of the cover glass. Now add a few drops of 0.001% Janus Green B solution at the opposite edge of the cover glass. Observe through your microscope that the mitochondria stain a blue color; then the blue-stained mitochondria lose their color in minutes as the result of the action of the enzymes (dehydrogenases) located on their surface. (c) How long did it require for the mitochondria to lose their color? ....

Variation in Cell Structure 4-2

Purpose To examine several kinds of cells and observe their similarities and differences.

Although all cells are basically similar in having protoplasm bordered by a plasma membrane, they vary considerably in structure, depending on the function they serve and the organism in which they are found.

A Part 1 EXAMINATION OF VARIOUS CELLS. Since plants lead a stationary existence, it is appropriate that their cells should have rather rigid cell walls for support. Animals, on the other hand, have to move around and need more flexible cells. In this Part you will study various types of cells to determine other differences between them.

Materials. Sprig of elodea; piece of potato; flower petals; human cheek cells; microscope; slides; cover glasses; microtome or razor blade; scissors; dropping pipette; colored pencils; toothpicks; iodine; methylene blue.

Procedure and observations. Section 1 *Cells of elodea.* Remove a fresh, green leaf from an elodea plant. Hold it in your hand a few minutes to warm it. Mount the whole leaf in a drop of water, and add a cover glass. Examine all areas of the leaf with the low power of your microscope. Then select a portion where the cells are clearly visible, center it in the field, and bring it into sharp focus under high power. Shift the focus with the fine adjustment to study the cells at various depths. The oval, green bodies in the cytoplasm are chloroplasts. They may be moving or streaming through the cell. (a) Can you see any moving chloroplasts? .... (b) If so, are they all moving in the same direction? .... (c) Are they all moving at the same rate? .... Chloroplasts do not have any structures for locomotion. (d) Account for their movement. ....

....

Draw a single cell in the space provided. Be sure that the chloroplasts are the correct shape and approximate number. If they were moving, indicate the direction with an arrow along the wall. Using a green pencil, shade the chloroplasts. Label: **cell wall, chloroplast, nucleus.**

Section 2 *Cells of the potato.* Cut a piece of potato about ¼ in. square and 1 in. long. Using a microtome or a razor blade, shave a piece off the end, making it as thin as possible. Prepare a wet mount of the thin piece and examine it under low power. (e) Describe the cells.

....

....

(f) Do you find any chloroplasts in the cells? .... Now add a drop of iodine at the edge of the cover glass and observe the cell as the iodine diffuses into it. Iodine turns blue-black in the presence of starch. (g) Is there starch in potato cells? .... (h) If so, where is it located? ....

Find a cell whose cell walls are visible and in which you can find numerous starch grains. Change to high power. Draw a cell, or a small group of them, in the space provided. Each cell should be about ½-1 in. in diameter. Using a blue pencil, color the starch grains. Label: **cell wall, starch grain.**

Cell of elodea leaf (H.P.)

Cell of potato (H.P.)

Section 3 *Cells of flower petals.* Remove a petal from several different flowers: a red one, a yellow one, a purple or blue one. Tear the petals into several pieces and prepare wet mounts of each one. Examine them under low and high power of the microscope. (i) Are the cells of all the flowers the same shape? .... (j) Are their cell walls the same thickness? .... (k) Do you find chromoplasts in the cells of all the flower petals? .... (l) If not, which flowers had chromoplasts in the cells? ....

(m) Are the chromoplasts moving? .... (n) Can you find additional pigments in the cells of the petals and, if so, where are they located? ....

Draw several cells of the flower petals in the space provided. Label: **cell wall, chromoplast, vacuole,** and **other pigments,** if present.

Flower petal cells (H.P.)

Section 4 *Human cheek cells (epithelial cells).* Gently scrape the inside of your cheek with a clean toothpick. Smear the material on a slide. Mount in water and add a drop of methylene blue and a cover glass. Examine under low power, noting the masses of cells and individual cells. Find the outer edge of the cytoplasm. (o) Compare it with the cell wall in plants. .... (p) What is it called? .... Study several different cells under high power. (q) Does the cell have a definite shape? .... (r) If so, what is its shape? .... (s) Is a central vacuole present? .... Reduce the light by closing the diaphragm. (t) Can you see the cytoplasm? ....

Draw several cells in a mass under low power, and a single cell under high power in the space provided. In the drawing from high power, label: **plasma membrane, cytoplasm, nucleus.**

Human cheek (epithelial) cells (L.P.)

Human cheek (epithelial) cells (H.P.)

Principles of Diffusion 5-1

Purpose To demonstrate diffusion of molecules of matter in various states.

All particles of matter are in constant motion, striking each other, rebounding, and spreading apart. The force causing this molecular activity is kinetic energy. If there is a concentration of molecules in a given space, the striking and rebounding increases, causing the molecules to disperse. We refer to this molecular dispersion as diffusion. Diffusion will continue until the molecules are dispersed as widely as possible and until they are concentrated equally in a space or a liquid system.

A Part 1 DIFFUSION OF GASES, LIQUIDS, AND SOLIDS. The physical state of a substance is determined by the kinetic energy of its molecules. In a *gas* molecules with high kinetic energy strike each other with great frequency and spread far apart. In a *liquid* molecules have less kinetic energy but still are in a state of motion. Further reduction in kinetic energy, resulting in little or no molecular movement, results in the dense molecular pattern we find in a *solid.* These molecular patterns influence the rate at which two substances that do not repel each other will diffuse into each other.

Materials. Peppermint oil or strong perfume; molasses; stop watch or interval timer; evaporating dish; two 250-ml beakers; yellow and blue pencils; potassium permanganate crystals.

Procedure and observations. Section 1 *Diffusion of gases.* To demonstrate diffusion of gases and the pattern and rate of flow of molecules during diffusion, prepare a chart of your laboratory or classroom in the space provided. Show the location of the teacher desk or demonstration table (in the front of the room) and all student stations. A member of the class will serve as the observer to record the time each member of the class detects the odor of diffusing molecules. A stop watch or interval timer should be used for timing the rate of molecular flow. The door and all windows should be closed to reduce air currents in the room.

At a given signal from the observer, the teacher will pour sufficient peppermint oil or strong perfume to cover the bottom of an evaporating dish. As each student detects the odor, he will raise his hand. The observer will record the elapsed time from pouring at the location of the student on the room chart. Continue until all members of the class have detected the odor of diffusing molecules. At the conclusion of the demonstration, class results will be transcribed to the room chart of each member of the class.

(a) What was the elapsed time between pouring and detection of the odor of diffusing molecules by the student nearest the evaporating dish? ....

Room chart

(b) by the student farthest from the dish? .... (c) Did the molecules diffuse through the air in the room at a uniform rate, as indicated by your chart? ....

(d) Explain why molecules of peppermint oil or perfume diffused from the dish into the air.

....

(e) How long will this diffusion continue? ....

(f) Was the diffusion of peppermint oil or perfume a one-way diffusion? ....

(g) Explain. ....

(h) What human factors among members of the class may have altered the results of your demonstration? ....

Oxygen and nitrogen are the principal gases composing air. Oxygen is heavier than nitrogen. (i) Why is diffusion necessary in maintaining organisms living in elevated environments?

....

Section 2 *Diffusion of liquids.* Substances also diffuse in liquids. To demonstrate this molecular diffusion, fill a 250-ml beaker half full of molasses syrup. Tilt the beaker and add water *slowly,* allowing it to run down the side. *Do not agitate the syrup in adding water.* Fill the beaker nearly to the rim, so that the water and molasses layers are approximately equal.

Observe the water and molasses layers closely. (j) Does the molasses appear to diffuse into the water? ....

(k) the water into the molasses? ....

(l) Taste the water at regular intervals and record your results. ....

....

....

(m) Compare the rate of molecular diffusion in liquids with that of gases. ....

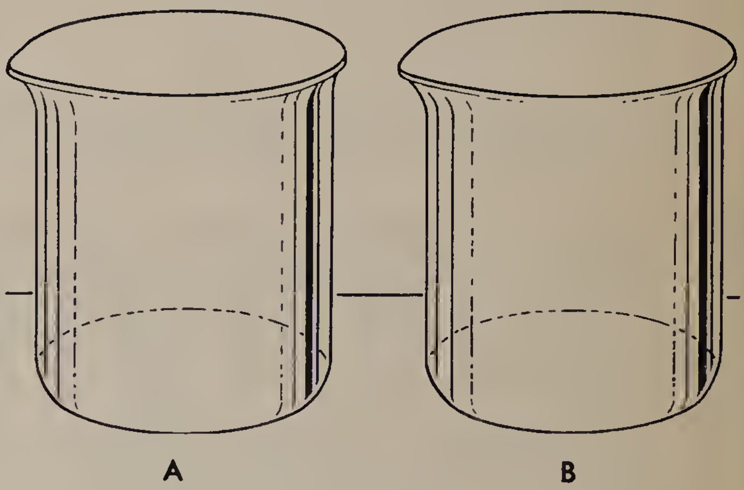


Fig. 5-1-a A Diffusion of water and molasses
B Diffusion of potassium permanganate crystals and water

Record the result of this demonstration in the beaker in Fig. 5-1-a, A. Use a yellow pencil to represent the molasses layer and blue to represent the water layer. Indicate molecular diffusion with arrows, using your lead pencil.

Section 3 *Diffusion of a solid and a liquid.* To observe diffusion of a solid and a liquid, fill a 250-ml beaker with distilled water (tap water may be used) and add several crystals of potassium permanganate. Watch the immediate result as the crystals settle to the bottom.

(n) What is the direction of flow of the purple color? ....

(o) Does the rate of diffusion of a solid appear to be as rapid as that of a gas? ....

(p) Are there indications that water molecules are diffusing into the potassium permanganate flowing from the crystals? .... (q) Does the volume of the solution increase as the crystals dissolve? ....

Illustrate the diffusion by showing water, crystals of potassium permanganate, and diffusion particles in the beaker in Fig. 5-1-a, B. Shade the water blue and draw the crystals and diffusing particles with your lead pencil.

Diffusion and Osmosis 5-2

Purpose To demonstrate diffusion across membranes and investigate the relation of membrane permeability to diffusion and osmosis.

In our study of diffusion as it relates to cells and living systems, we are concerned primarily with the movement of water and substances dissolved in water. The most important factor affecting diffusion in these water solutions is molecular concentration.

A Part 1 **DIFFUSION ACROSS MEMBRANES.** Since living cells are bounded by membranes, we are especially concerned with the diffusion of water and dissolved substances across membranes. In this Part you will demonstrate diffusion through a permeable membrane and a special type of diffusion across a differentially permeable membrane, known as *osmosis*.

Materials. Two osmosis demonstration apparatus or 2 double thistle tubes; 2 battery jars 4 × 5 in. or larger; 2 ring stands; 4 tube clamps (to fit ring stands); fine muslin; osmosis membrane (animal membrane, parchment, or cellophane); string or rubber bands; glass-marking and lead pencils; molasses (syrup).

Procedure and observations. Cut a piece of muslin large enough to cover the mouth of a thistle tube and extend at least a half-inch beyond the rim. Hold the bulb up and, with a finger pressed against the opening at the other end of the tube, fill the bulb with molasses syrup. While you are holding the tube, have another student put the muslin over the mouth of the bulb. Fold the edges down and fasten the muslin securely around the rim, using string or rubber bands. Fasten the thistle tube to the clamps on the ring stand with the molasses-filled bulb down, as shown in Fig. 5-2-a. Fill the battery jar with water and submerge the molasses-filled bulb.

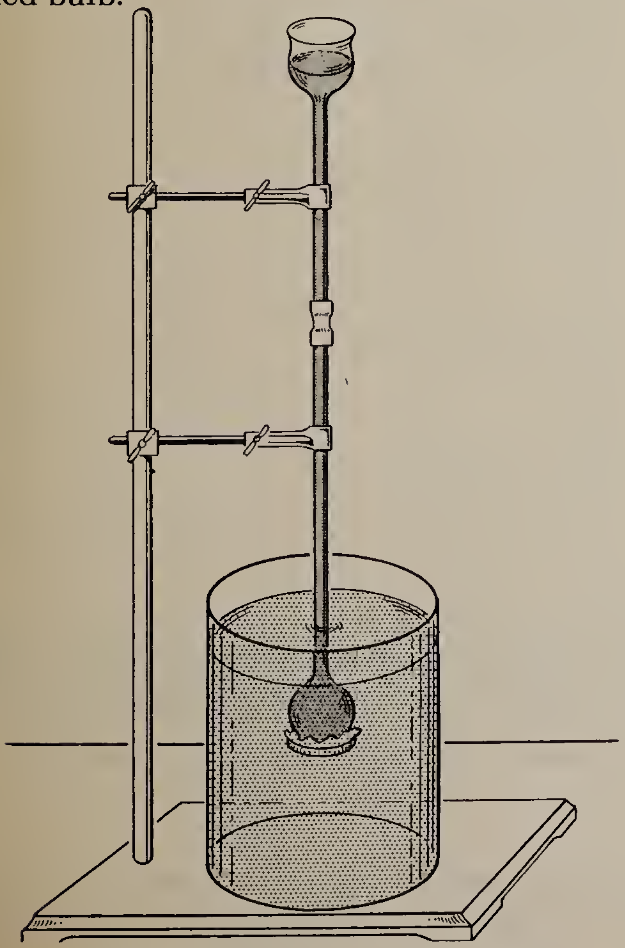


Fig. 5-2-a Diffusion apparatus

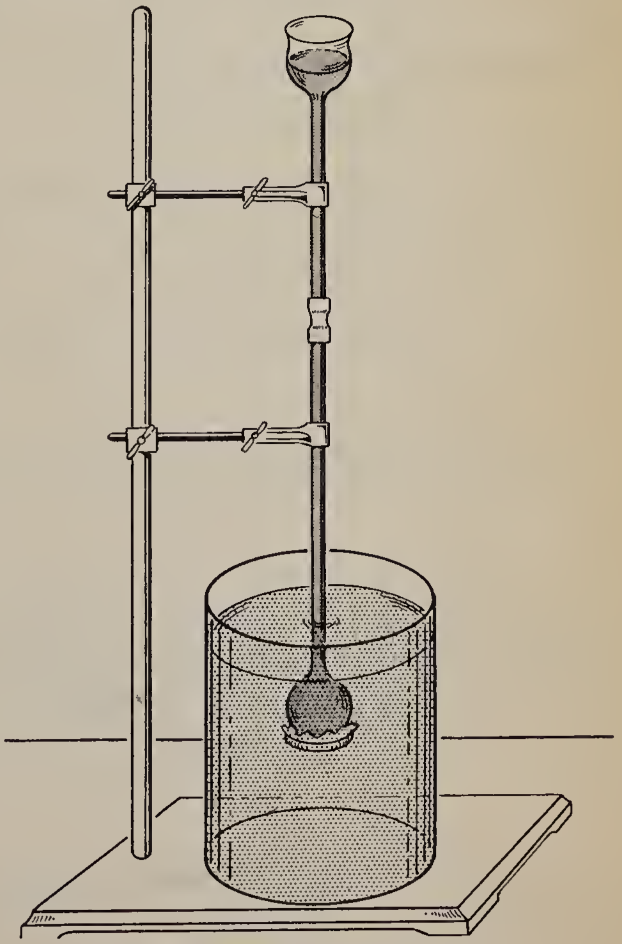


Fig. 5-2-b Osmosis apparatus

Prepare the second setup as you did the first, but substituting an osmosis membrane for the muslin. It may be necessary to soak this membrane in water for several minutes to make it pliable before fastening it over the mouth of the thistle tube.

Use a glass-marking pencil to mark the level of the molasses in both setups at the start of the experiment. Use a lead pencil to draw this line on the drawings of the apparatus in Figs. 5-2-a and 5-2-b and indicate it as *Start*. Examine the apparatus at the close of school and twice each day for 24 or 48 hours. Mark any change in the level of the solutions on the thistle tubes and on the drawings of the setups. Indicate the elapsed time in hours from the start of the demonstration at each level marked on the drawings. At the close of the experiment, shade the solution in the drawings of the thistle tubes to show the highest level reached. Indicate diffusion of molasses into the water in the battery jar by shading.

(a) Was the region of greatest diffusion pressure of water molecules in the battery jar or in the thistle tube at the start of both demonstrations? ....

(b) Where was the region of greatest diffusion of sugar (molasses) molecules? ....

(c) Is there evidence of diffusion of water through the membrane in A? ....

(d) in B? .... (e) Is there evidence of diffusion of sugar molecules through the membrane in A? .... (f) in B? .... (g) On what evidence do you base this conclusion? ....

....

(h) Are water molecules diffusing through the membrane in both directions and at the same rate in A? .... (i) in B? .... (j) Was a state of equilibrium reached in A? .... (k) in B? .... (l) What condition produces a state of equilibrium?

(m) Explain why the molasses solution rises in one tube but not in the other. ....

....

(n) What condition in B caused water diffusion to stop? ....

....

(o) How did the membranes illustrate differences in permeability? ....

....

(p) In terms of what you observed in B, give a definition of osmosis. ....

....

B Part 2 OSMOSIS DEMONSTRATION USING AN EGG. The membrane lining the shell of an egg is differentially permeable and, therefore, similar to the osmosis membrane you used in Part 1. In using an egg to demonstrate osmosis, it is necessary to bring the membrane in contact with water. This requires removal of a portion of the shell without damage to the membrane.

Materials. Egg; forceps; dissecting needle; probe; 8-in. battery jar; 250- or 400-ml beaker; wire triangle, 2½ in. side; ring stand; glass tubing, 5-6-mm. diam., tube clamps, 3 ft. or longer; paraffin; small brush; glass-marking pencil; dil. HCl.

Procedure and observations. You may use either of two methods for removing a portion of the shell of an egg to expose the membrane lining the shell. One method is to chip out a circular opening in the rounded end of the egg. This end usually contains an air cell in which the membrane lies below the shell. You may locate the air cell by holding the egg close to a light bulb, rounded end up. Carefully, pierce the shell with the point of a dissecting needle. Chip out small pieces of the shell to enlarge the opening to a diameter of one-half to three-quarters of an inch. Use care not to pierce the membrane. The slightest tear or hole in the membrane will prevent the demonstration from working.

A second method is dissolving of a portion of the shell in dilute hydrochloric acid. To do this, pour the acid in a small dish or beaker and submerge the end of the egg. A circular area of the shell about 1 in. in diameter should be dissolved. While dissolving the shell, check the egg frequently to avoid damage of the membrane. This should be done by removing the egg, rinsing it, and pressing the shell lightly. The end of the egg should be pliable when the shell is sufficiently dissolved. When the rounded end of the egg is prepared, drill a hole through the shell and membrane at the opposite end, using a dissecting needle. Enlarge the hole with a probe to the diameter of the glass tubing you will use.

Put a battery jar on the base of a ring stand and set a 250- or 400-ml beaker in the center of the jar. Put a triangle on the rim of the beaker, as shown in Fig. 5-2-c. It may be necessary to bend down the wire ends of the triangle to fit it in the battery jar. Place the egg, round end down, on the triangle. Fasten a length of glass tubing to clamps attached to the upright rod of the ring stand. Use two or more clamps to hold the rod securely. Carefully, lower the glass tubing to the hole in the egg and insert the end of the tube just below the shell. Pour melted paraffin around the tube to form a watertight seal between the glass and the shell. You may want to apply the paraffin with a small brush. Pour sufficient water into the battery jar to submerge the egg.

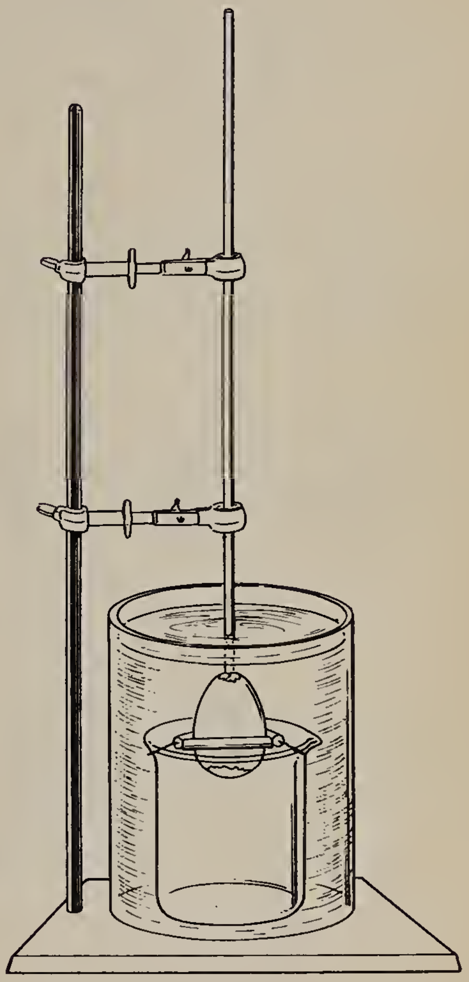


Fig. 5-2-c Osmosis demonstration using an egg

Check the level of the egg content and water in the tube twice each day for two or three days, or until the column reaches its greatest height. It may be necessary to extend the glass tube by joining a second section with a piece of rubber tubing. Use a glass-marking pencil to mark the level of the liquid on the tube each time the apparatus is checked.

(a) What was the highest level reached by the liquid in the tube? ....

(b) Why did water diffuse into the egg? ....

(c) Did egg content diffuse into the water in the battery jar? .... (d) At what point did water stop diffusing into the egg? ....

Interpretation. Discuss your results in relation to permeability of the shell membrane of an egg and in terms of concentration of water and egg content. ....

Diffusion Through Cell Membranes 5-3

Purpose To investigate the passage of molecules and ions through differentially permeable membranes.

One of the important functions of the cell membrane is to regulate what enters and leaves the cell. Water molecules as well as molecules and ions of many other substances dissolved in water pass in and out of the membrane. Other molecules and ions are normally retained within the cell, while still others are normally rejected. This selective control is extremely important in the life of the cell.

A Part 1 DIFFUSION THROUGH LIVING CELL MEMBRANES. Osmosis is the diffusion of water through a differentially permeable membrane from an area of greater concentration to one of lesser concentration. In this Part you will observe the changes that occur in cells when the water concentration is altered.

Materials. Sprig of elodea; potato; flower petals (purple petunia or lobelia); microscope; slides; cover glasses; dropping pipette; 2 culture dishes; scalpel or knife; 10-ml graduate; test-tube holder; filter paper; distilled water; conc. NaCl sol'n; 15% NaCl sol'n; 15% sugar sol'n; dil. HCl; dil. NH_4OH.

Procedure and observations. Section 1 *Diffusion through elodea leaf cell membranes.* Remove a leaf from a sprig of elodea and mount it whole in a drop of water on a microscope slide. Add a cover glass and examine with high power. (a) Describe the position of the cell membrane and the chloroplasts. ....

....

.... Draw a cell in the space provided.

Using a dropping pipette, put one drop of a 15% salt solution at the edge of the cover glass. Continue adding salt solution a drop at a time until a change occurs in the appearance of the cell. (b) How many drops were added before a change occurred? ....

(c) Describe the change in the appearance of the cell. ....

....

.... (d) Judging from the appearance of the cell, would you say that water moved into or out of the cell? ....

(e) What condition in the cell environment caused this change? ....

....

.... Draw a cell in the 15% salt solution in the space provided.

Put a piece of filter paper or the edge of a paper towel at the edge of the cover glass and absorb as much of the salt solution as possible. Then add distilled water, a drop at a time, at the edge of the cover glass, watching the cells closely as each drop is added. (f) Describe any change that occurs in the cell content. ....

....

....

Draw a cell flooded with distilled water in the space provided.

Elodea cell in tap water   Elodea cell in 15% salt solution   Elodea cell in distilled water

Section 2 *Turgor and loss of turgor in a plant tissue.* To observe the relation of water diffusion to turgor and loss of turgor in plant cells, fill one culture dish with clear water and a second dish with a concentrated sodium chloride solution. Cut six slices of potato about one-quarter inch thick. Put two slices in the clear water and two in the concentrated salt solution. Leave the slices in the dishes 15-30 minutes. Examine the remaining two freshly cut slices. (g) Describe their texture and stiffness. ....
Remove the slices from clear water and examine them. (h) Compare the texture and stiffness of these slices with the freshly cut slices. ....

(i) Has the turgidity of the cells in these slices increased or decreased? ....

(j) Account for this change in terms of water diffusion. ....

....

.... Remove the slices from the concentrated salt solution and examine them. (k) Compare their texture and stiffness with the slices in clear water. ....

(l) with the freshly cut slices. ....

(m) Have the cells of the slices in salt solution gained or lost turgor? ....

(n) Account for this change in terms of water diffusion. ....

....

....

Section 3 *Diffusion into cells of colored flower petals.* In this Section, you will determine the permeability of living membranes to H^+ ions of an acid and the OH^- ions of a base. Cells of many flower petals contain a pigment that acts as an indicator. In the presence of H^+ ions it is one color, but in the presence of OH^- ions, changes to another color. By observing these color changes, you can determine that H^+ or OH^- ions passed across the membrane and entered the cell.

Prepare microscope slides of portions of three purple petunia or lobelia flower petals (other purple or blue flowers may be substituted). Mount one petal in distilled water, one in dilute hydrochloric acid, and the third in dilute ammonium hydroxide. Add cover glasses and watch for changes in color of the flower petals. (o) Describe the appearance of the cells in distilled water. ....

....

(p) Describe the cells treated with hydrochloric acid. ....

....

(q) Describe the cells treated with ammonium hydroxide. ....

.... (r) In what way have you demonstrated diffusion?

....

....

....

A Part 2 DIFFUSION THROUGH KILLED MEMBRANES. Living membranes normally retain the cell contents, including pigment molecules. In this Part of the Investigation you will observe what happens to this control when a cell membrane is killed.

Materials. Fresh beet; package of yeast (dried); microscope; slide; cover glass; 2 test tubes; test-tube holder; two 660-ml beakers; Bunsen burner; distilled water; congo red solution.

Procedure and observations. Section 1 *Permeability of living and killed beet cell membranes.* Cut several slices of fresh beet, wash them, and put them in a beaker of distilled water. Put another set of washed slices in a beaker of distilled water and boil two to three minutes. Allow both beakers to stand 5-10 minutes, then examine the water in both. (a) Describe the appearance of the cold water. ....

(b) Describe the water in which the beet slices were boiled. ....

.... (c) Account for the differences in color of the water in the two beakers. ....

....

....

Section 2 *Diffusion of pigment molecules into yeast cells.* Using dry yeast, prepare a suspension in 500 ml of water. (This quantity of suspension will be sufficient for the entire class.) Fill two test tubes half full of the yeast suspension. Add four drops of congo red solution to both tubes. Allow one tube to remain at room temperature for 5-10 minutes. Heat the other tube in the flame of a Bunsen burner until the suspension boils. When the boiled suspension has cooled, prepare microscope slides of both suspensions and examine the cells under high power.

(d) Describe the cells in the unboiled suspension. ....

....

(e) Describe the cells in the boiled suspension. ....

....

(f) Account for the difference in the appearance of the yeast cells. ....

....

....

B Part 3 DIFFUSION THROUGH THE MEMBRANE OF AN EGG. In the demonstration of osmosis in Investigation 5-2, you observed the passage of water through the differentially permeable shell membrane of an egg. In this demonstration, water movement was largely into the egg, resulting in dilution of the egg content and forcing of the content into a tube. In this Part you will observe water diffusion through an egg membrane in two directions, as determined by the concentration of water molecules in external fluids. Increase and decrease of the egg volume will indicate the direction of water passage across the membrane.

Materials. Two eggs; microscope; slide; cover glass; glass rod or spoon; three 250-ml beakers; two 600-ml beakers; 50-ml graduate; distilled water; dil. HCl; 15% salt solution; congo red solution.

Procedure and observations. Dissolve the shells of two eggs by pouring dilute hydrochloric acid over them in a beaker. The dissolving may require several hours, during which the eggs should be checked with a glass rod or spoon. (CAUTION: *In checking the eggs, use care not to spill or splash the hydrochloric acid.*) When the shells are dissolved and the eggs are pliable, wash them in cold water, using care not to rupture the membrane.

It will be necessary to determine the volume of both eggs at the beginning of the experiment and several times in the course of the experiment. This can be done by means of water displacement. Fill a 250-ml beaker to the rim with water and put it in a 600-ml beaker. Carefully place an egg into the water in the 250-ml beaker. This will cause an amount of water equal to the volume of the egg to overflow into the 600-ml beaker. Remove the smaller beaker containing the egg and pour the water that overflowed into a 50-ml graduate and measure it accurately. Record this volume of water displacement for both eggs in the table below.

Submerge the first egg in a beaker of distilled water and the second in a beaker of 15% salt solution. On the second day, measure the volume of both eggs by water displacement and note whether there is an increase or decrease in volume and any changes in the appearance of the eggs. Record all data in the table provided.

Day	*Distilled Water*			*15% Salt Solution*		
	Volume by water displacement	*Change in volume*	*Change in appearance*	*Volume by water displacement*	*Change in volume*	*Change in appearance*
1						
2						
3						
4						

Interpretation. In terms of water concentration inside and outside the shell membrane, explain the changes in volume and appearance of the two eggs, as recorded in your table of observations. ....

....

....

....

....

C Part 4 WATER BALANCE IN A ONE-CELLED ORGANISM

Problem. How does a one-celled organism living in water maintain a balance with its environment?

Since water passes freely through cell membranes by osmosis, an organism whose cell surface is in constant contact with fresh water must have a mechanism for eliminating excess water. Otherwise internal pressure would build up and the cell would burst. The paramecium, a one-celled organism you will study in detail in Unit Three of MODERN BIOLOGY, has two organelles called contractile vacuoles for water elimination. A study of these structures in living paramecia provides an excellent opportunity for understanding water regulation in living things.

Suggested materials. Culture of paramecium (either *Paramecium caudatum* or *Paramecium multimicronucleatum* are good); culture slide and cover glass for hanging drop; micropipette; microscope; methyl cellulose (for quieting the organisms); dropping bottles containing distilled water; 0.25, 0.50, 0.75, and 1.00% aqueous solutions of NaCl.

Suggested procedure. Prepare a hanging drop containing several organisms from your culture. Add a small drop of methyl cellulose to slow the paramecia down. Find one cell that is not moving and examine it closely with low power and high power. Notice especially the form of the contractile vacuoles and the way they fill and contract in water elimination.

Time the interval between contractions of one of the vacuoles for 10 or more times and determine the average. Is the rate of filling and contraction fairly constant? Check several paramecia and determine if this rate varies among different individuals. Since the paramecium contains two vacuoles, it will be interesting to observe the relation of these vacuoles to one another. Do they contract in unison or alternately? Is the interval between contractions uniform?

What will happen to the rate of water elimination if the water environment of the paramecium is altered? You can use the salt solutions suggested in the list of materials to determine how they affect the rate of contraction of the contractile vacuoles. The hanging drops should be made of each solution and the paramecia added with a micropipette so as not to alter the known concentration of the salt solutions.

Analysis and conclusions. Your data on the rate at which contractile vacuoles contract under different conditions should be summarized in a table and a line graph. Your study should result in an understanding of the way the contractile vacuoles in the paramecium operate to maintain water balance in the face of changing conditions.

Characteristics and Composition of Chlorophyll 6-1

Purpose To extract chlorophyll and observe its characteristics and composition.

Chlorophyll, contained in chloroplasts in green plant cells, is the substance that makes photosynthesis possible. Sunlight causes its electrons to move to higher energy levels, or shells, In this excited state the chlorophyll molecule acts as an energy carrier, releasing its energy to split water in the photo phase.

A Part 1 **EXTRACTION AND TESTING OF CHLOROPHYLL.** In this Part you will become familiar with a technique for extracting chlorophyll and with some of its characteristics.

Chlorophyll has the ability to fluoresce. This means that as it absorbs radiation from some other source, such as sunlight, it emits radiation in the form of visible light. Biologists have found that this property of fluorescence is related to the role of the chlorophyll molecule as an energy carrier.

Materials. Fresh leaves of geranium or begonia (frozen spinach may be used); Bunsen burner; tripod; water bath; 600-ml beaker; 2-oz. square bottle; 300-ml ground glass bottle with stopper; 250-ml beaker; 2 × 2 projector or other light source; green food coloring; 95% ethyl alcohol; carbon tetrachloride.

Procedure and observations. Put the leaves in boiling water for two minutes. (a) Does the pigment in the leaves dissolve? .... (b) Explain your answer. ....

....

Transfer the wilted leaves to a beaker containing 300 ml of 95% ethyl alcohol and set the beaker in a water bath or pan of hot water. (CAUTION: *Do not boil alcohol directly over a flame*). Boil the leaves in the alcohol for five minutes. (c) Does the pigment in the leaves dissolve? .... (d) Explain your answer. ....

.... Continue to add wilted leaves to the alcohol until you obtain a dark green solution.

Allow the solution to cool. Pour some of it into a 2-oz. square bottle until it is almost full. Then put 100 ml of the solution into a 300-ml ground glass bottle. Put the stopper in it and set it aside to be used later.

Place the square bottle with the chlorophyll solution in a direct beam of light from a strong light source. Look through the open neck of the bottle at the surface of the solution.

(e) What do you see? ....

(f) What property of chlorophyll does this demonstrate? ....

....

Add green food coloring to some alcohol until the color is similar to that of the chlorophyll solution. Put the green food coloring solution in a 2-oz. square bottle and place the bottle in the strong beam of light. Look through the open neck of the bottle at the surface of the solution.

(g) What do you see? .... (h) Compare the results you obtained with those for the chlorophyll solution. ....

....

Although chlorophyll is the dominant pigment in most plant parts, you know from your reading that other pigments are present. To determine whether the chlorophyll solution you have prepared is composed entirely of chlorophyll, you can utilize the principle that a substance may be more soluble in one liquid than it is in another.

Add 130 ml of carbon tetrachloride to the solution you put in the bottle. (CAUTION: *The fumes of carbon tetrachloride are poisonous. Do not inhale them or spill any of the liquid on your skin.)* Put the stopper in the bottle and shake the solution. (i) Study the contents of the bottle and describe what happened. ....

(j) Which pigment is more soluble in alcohol? ....

(k) in carbon tetrachloride? ....

B Part 2 SEPARATION OF CHLOROPHYLL EXTRACT BY PAPER CHROMATOGRAPHY. In Part 1 you separated pigments in chlorophyll extract by using two different solvents—alcohol and carbon tetrachloride. Another method is called paper chromatography. The technique involves putting a spot or line of the extract on a piece of filter paper, allowing it to dry, and then adding a solvent, which moves along the paper by capillary action. The different substances dissolved in the mixture are carried along at different rates and separated out along the filter paper. The result is called a chromatogram. In this Part you will make two kinds of chromatograms.

Materials. Chlorophyll solution; paper clip; test tube; fine-tipped pipette; Petri dish; cork stopper for test tube; filter paper; pencil; acetone; petroleum ether.

Procedure and observations. To prepare the setup for column chromatography, Fig. 6-1-a, cut a piece of filter paper one-half inch wide and long enough to extend from the cork stopper almost to the bottom of the tube. Insert a paper clip on the under surface of the cork stopper and attach the filter paper to the paper clip. Pour sufficient solvent (5 ml acetone and 5 ml petroleum ether) into the test tube so that the bottom of the filter paper will extend into the solvent.

Using a pencil, draw a line across the paper approximately two inches from the bottom of the paper. Using a fine-tipped pipette, spread a drop of the pigment solution along the line. Allow it to dry. Then spread another drop of the solution along the pencil line and allow to dry. Continue to add the solution until the line is almost black.

Put the filter paper in the test tube containing the solvent. The stopper should fit tightly because the solvents are volatile. Be sure the tip of the paper is in the solvent, but not touching the sides of the tube. Observe the separation of the pigments until the solvent has almost reached the top. Since the solvent evaporates rapidly and the pigments are often unstable and fade or change color, you *must* record your observations immediately. Mark on the filter paper the point to which the solvent flowed, and the position and color of the various pigment spots. (a) How many color zones did you have on your chromatogram? ....

(b) List and describe the color zones from top to bottom.

....

....

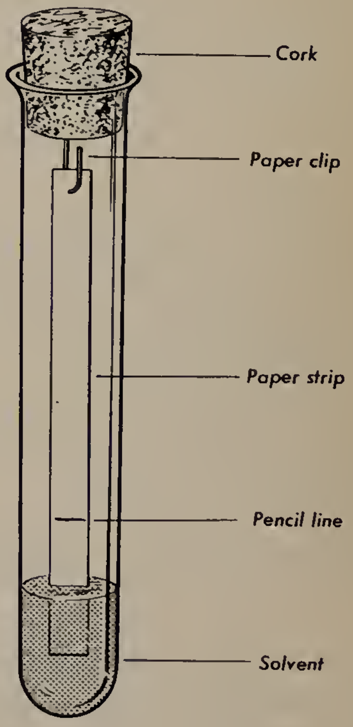


Fig. 6-1-a Setup for column chromatography

The yellow-orange pigments are the carotenes, the yellow-green are xanthophylls, the dark green one is chlorophyll *a*, and the blue-green one is chlorophyll *b*. (c) Are the color zones all the same width? .... (d) Account for your answer. ....

....

To verify the results, use the *disk* chromatography technique shown in Fig. 6-1-b. Cut a wick one-eighth inch wide in a piece of three inch filter paper. Fold the wick down. Put the chlorophyll, a drop at a time, along the fold. Put the acetone and petroleum ether solvent into the bottom of a Petri dish. Put the filter paper disk with the chlorophyll solution on the Petri dish, allowing the wick to extend into the solvent. Observe the separation of the pigments that occurs in rings around the filter paper. Identify the position and color of the various concentric rings on the paper, beginning with the outer ring.

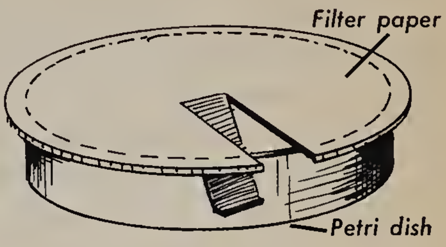


Fig. 6-1-b Setup for pigment separation

Interpretation. Summarize the principles employed in paper chromotography and the data you gained from using the technique in analyzing chlorophyll. ....

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C Part 3 ABSORPTION OF LIGHT BY A CHLOROPHYLL SOLUTION

Problem. What wavelengths of light are absorbed by a chlorophyll solution?

The visible portion of the radiation from the sun, which we see as white light, is composed of rays of varying wavelengths and energy levels. When these radiations are separated by bending light through a prism, they appear as the colors of the rainbow. We refer to this as the visible spectrum. Violet appears at one end of the spectrum, passing into blue, green, yellow, orange, and red. The wavelength of each color is measured in millimicrons (mμ). One millimicron is one-thousandth of a micron, one-millionth of a millimeter, or about one 25-millionth of an inch. The wavelength at the violet end is about 400 mμ. Wavelengths increase through the other colors of the spectrum to about 760 mμ at the red end. As the wavelengths increase, the energy level of the radiation decreases. Thus, violet radiation has the shortest wavelength and the highest energy level of the visible spectrum, while red has the longest wavelength and the lowest energy level.

Since light supplies the energy absorbed by chlorophyll in photosynthesis, biologists are interested in the amount of energy absorbed from the various wavelengths of the spectrum. Are all wavelengths absorbed equally, or are some absorbed more than others, while some are reflected and still others pass through the chlorophyll? The fact that chlorophyll is green would indicate that at least part of this wavelength is reflected.

You can determine which wavelengths are reflected or absorbed and which ones pass through a chlorophyll solution by means of a spectrometer. This instrument separates light into the visible spectrum by bending the radiations through a prism. How is the appearance of the spectrum altered as light passes through a chlorophyll solution?

Suggested materials. Chlorophyll solution; 250-ml beaker; 6-in. culture tube (or test tube); ring stand; clamp to hold culture tube; white light source (long wire line filament blub); spectroscope.

Suggested procedure. Before using the chlorophyll solution, you should become familiar with the spectroscope. This instrument consists of a collecting tube which is directed toward a light source, a prism which bends the radiations and forms the visible spectrum, and a telescope tube through which you view the spectrum. If the prism is exposed, it may be necessary to cover this portion of the instrument with a piece of dark cloth. A white light source produced by a line filament (long wire) bulb is excellent for use with a spectroscope. Otherwise, the collecting tube may be directed toward daylight. Examine white light and study the color bands of the visible spectrum. Note carefully the width of the bands and the manner in which one color passes into another. List the order in which the colors appear.

When you are familiar with the spectroscope, prepare a fresh chlorophyll extract, as directed in Part 1 (or use remaining untreated chlorophyll extract). Fill a 6-in. culture tube or test tube with the chlorophyll extract. Mount the tube in a clamp and fasten it to a ring stand. Adjust the tube so that it is in line with the collecting tube of the spectroscope, and between the light source and the collecting tube. Examine the radiations that appear in the spectroscope after passing through the chlorophyll solution. Only those radiations that pass through the chlorophyll will appear as bands of color in the spectroscope. Radiations that are absorbed or reflected will not appear. Instead, dark bands and narrowed bands of color will be seen.

You may need to examine each portion of the spectrum in white light and in light passing through the chlorophyll solution several times to determine the relative amount of absorption or reflection of each wavelength of radiation. Estimate the amount of each radiation absorbed or reflected as 25 percent, 50 percent, 75 percent, or 100 percent. You may present your data in several ways. One method is a graph. Plot the wavelengths of radiations along the horizontal axis, indicating violet-blue radiations as 400-500 mμ, green-yellow as 500-600 mμ, and orange-red as 600-700 mμ. Indicate your estimate of the percent of absorption or reflection of each radiation on the vertical axis. You may also prepare a table of results, or use colors to show the appearance of the visible spectrum in white light and in light passing through a chlorophyll solution.

Analysis and conclusions. Based on your experimental data, discuss the absorption of light by a chlorophyll solution and its significance in terms of photosynthesis. Indicate the degree of absorption of each wavelength of radiation. Since the spectroscope does not distinguish between absorption and reflection of radiations, it will be necessary to consider the possible amount of reflection of radiations from the chlorophyll.

Purpose To observe photosynthesis under controlled conditions in order to determine requirements for the process, factors that influence its rate, and products formed.

The synthesis of carbohydrates in certain chlorophyll-containing cells requires specific inorganic raw materials and light energy to fuel the process. Furthermore, various conditions in the environment have a direct influence on the rate at which photosynthesis occurs. You can demonstrate the chemical requirements, energy requirements, and environmental influences by observing the process in the laboratory under controlled conditions.

A Part 1 DEMONSTRATION TO SHOW THAT LIGHT AND CARBON DIOXIDE ARE REQUIRED FOR PHOTOSYNTHESIS. Living green plant cells can synthesize carbohydrates in the presence of absorbed light if there is sufficient supply of water and carbon dioxide. However, if light energy is not available, photosynthesis does not occur.

In this Part you will test for the presence of starch to determine whether photosynthesis can occur in the absence of light or carbon dioxide.

Materials. Four healthy green geranium or coleus leaves; 4 small jars or 50-ml beakers; 4 glass containers with lids; 250-ml beaker; Bunsen burner; tripod; water bath or pan; 5% baking soda solution; iodine; alcohol (70-100%).

Procedure and observations. Select four healthy green geranium or coleus plants and remove a young, vigorous leaf from each of them.

Put the leaves in separate small bottles or beakers of water. Number the glass containers with caps 1-4. Put a bottle containing a green leaf in each numbered container. See Fig. 6-2-a for setup.

Now you are ready to add the reagents. Avoid touching the leaves with the reagents. Close the jars tightly after the reagent is added. To jars 1 and 3, add a 5% baking soda solution to a depth of about one inch. Add water to a depth of one inch in jars 2 and 4. Put jars 1 and 2 in a strong light source (natural light is best). Put a 100-watt bulb two feet from the plants to supply a light source throughout the night, or if possible, for 48 hours. Put jars 3 and 4 in a dark cupboard. After 48 hours, remove the leaves from their individual containers. Now you are ready to test for starch production.

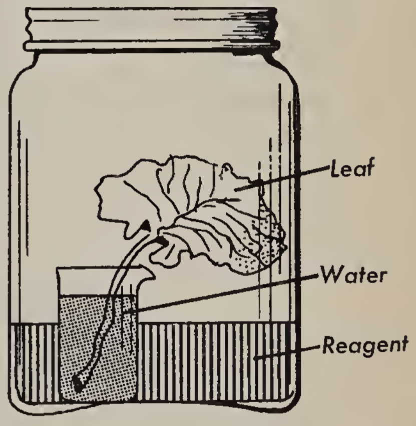


Fig. 6-2-a Setup for photosynthesis demonstration

Dip a leaf into a pan of boiling water to kill the protoplasm in the leaf cells. Put it into a beaker of alcohol and set the beaker in a water bath or a pan of hot water. *(Do not boil alcohol directly over a flame.)* Boil the leaf until it is colorless. You have then removed all of the chlorophyll from the leaf. Dry the leaf on a piece of paper towel and flood it with iodine. Observe any change in the leaf. Record the change in the table provided. Determine whether the starch test for each leaf is positive or negative and record the results in the table. Determine which factor or factors were missing that prevented the production of starch by the tissues and record these in the table.

(a) Why was it necessary to remove the chlorophyll before making the starch test?

....

Leaf	*Effect of iodine on leaf tissues*	*Starch test*	*Factor missing*
1			
2			
3			
4			

A Part 2 **DEMONSTRATION TO SHOW THAT OXYGEN IS GIVEN OFF DURING PHOTOSYNTHESIS.** Biologists have found that during the light, or photo, phase of photosynthesis the water molecules are split and oxygen is set free.

Water plants are used in this Part because any gas given off appears as bubbles in the water. You can collect the gas in a test tube or other container by directing the bubbles into an inverted, water-filled container. The gas will displace the water.

Materials. Elodea; ring stand; funnel (3-6 in., short stem); tube clamp; section of glass tubing (6 in. long); cork or rubber stopper; splint.

Procedure and observations. Put a bunch of elodea or other water plant in a battery jar filled with water. Cover the plants with a funnel 3-6 in. in diameter. Plug one end of a piece of glass tubing 6 in. long and 1 in. in diameter with a cork or rubber stopper. Fill it with water and invert it over the funnel stem below the surface of the water (see Fig. 6-2-b). Be careful not to leave any air in the tube. Set the experiment in a sunny place and observe for several days. (a) Why is it necessary to place the plant in a sunny place? ....

....

(b) What is the role of the energy in the light phase of photosynthesis? ....

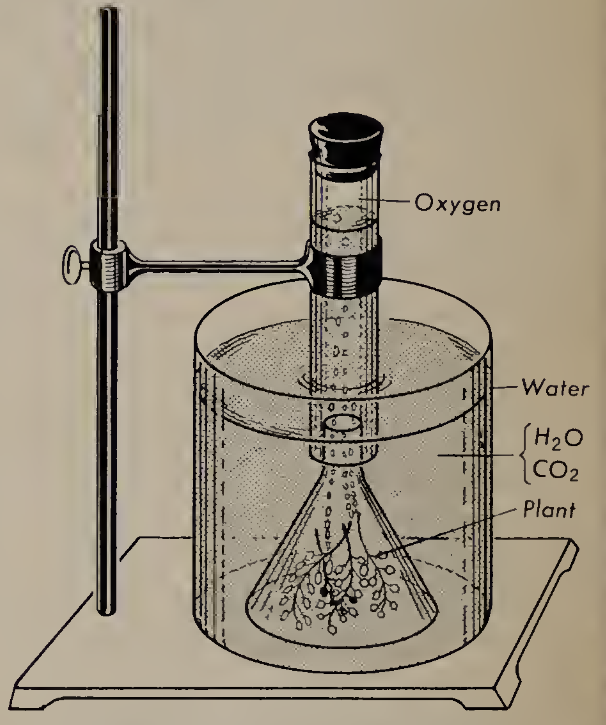


Fig. 6-2-b Test for oxygen

When the tube is partially filled with gas, remove the stopper and test the gas with a glowing splint. (c) Describe what happened. ....

(d) If oxygen was present, where did it come from? ....

A Part 3 **DEMONSTRATION TO SHOW THAT GLUCOSE IS PRODUCED IN A GREEN LEAF.** Glucose is a soluble nutrient which does not remain in the leaf, but is converted to starch. The amount of glucose produced in the leaf varies with the kind of leaf and is influenced by environmental factors. You can use the same chemical test for determining the presence of glucose in leaves that you used in determining its presence in organic nutrients.

Materials. Green leaves; mortar; pestle; ring stand; culture tube; test-tube holder; Bunsen burner; 250-ml beaker; tripod; Fehling's solution A and B.

Procedure and observations. Pick several leaves from a tree or other plant. Grind the leaves in a mortar. Put the ground leaves in a beaker or dish and cover with warm water. Stir for several minutes. Pour some of the water and ground leaves into a culture tube (about one-quarter full). Then add equal amounts of Fehling's solution A and B to fill the tube about half full. Heat gently to boiling and continue boiling for a few minutes. A yellowish color indicates a weak glucose test, while a brick-red color is a strong test. (a) Is your test for glucose weak, strong, or negative? .... Pick some leaves from several kinds of trees in your vicinity (you may wish to use several species of a single genus) and compare the amount of glucose produced by each. (b) Do all the leaves show the same degree of glucose production? .... (c) Account for your results. ....

....

....

B Part 4 MEASURING THE RATE OF PHOTOSYNTHESIS UNDER VARYING ENVIRONMENTAL CONDITIONS. How fast does photosynthesis take place? You can determine the rate of photosynthesis by counting the number of oxygen bubbles that escape from the cut end of a sprig of elodea during a five-minute interval. Recall that you identified oxygen as the by-product of photosynthesis in Part 2 of this Investigation.

Materials. Elodea; 8 culture (test) tubes; eight 2-in. pieces of glass rod; slide projector or other strong light source; water bath; centigrade thermometer; Bunsen burner; ice; sodium bicarbonate.

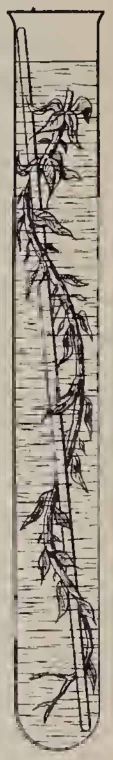


Fig. 6-2-c Rate of photosynthesis apparatus

Procedure and observations. To determine the effect of light on the rate of photosynthesis, fill four culture tubes to within an inch of the top with water. Select four sprigs of elodea that have been growing in bright light and previously cut under water to a length of about three inches. Tie the tip of each sprig to a 2-inch piece of glass rod. Now put each elodea plant in a separate culture tube. The weighted end will settle to the bottom and hold it in place (Fig. 6-2-c). Add a pinch of sodium bicarbonate to each tube to charge it with carbon dioxide. Put one tube in direct sunlight or bright light (projector); a second in daylight; the third in diffuse light; and the fourth in a cupboard. Leave the tubes under the various light conditions for fifteen minutes. Then observe the cut end of the plants carefully. Using a timer or a watch with a second hand, count the number of bubbles given off by each of the plants in one minute. Record the result in the table. Then continue to count the bubbles for five minutes and record an average number of counts per minute.

Environmental condition	*Rate per minute*	*Rate per five minutes*	*Average rate per minute*
Sunlight or projector			
Daylight			
Diffuse light			
Darkness			

(a) Account for the variation in rate of photosynthesis in the four plants. ....

....

....

You can also determine the effect of temperature on the rate of photosynthesis by using elodea plants. Prepare four specimens as you did for the exercise on light intensity and its effect on photosynthesis. Place one tube in a water bath and using ice chips reduce the temperature to 10°C. Allow the tube to stand in the water bath until the oxygen bubbles are given off at a regular rate. Count the bubbles that are given off in one minute. Continue to count the bubbles for five minutes and record an average number of bubbles for a minute.

Adjust the temperature to 20°C by heating and put a second tube with an elodea plant in the water bath. Allow it to adjust to the water temperature; then count the bubbles produced and record as you did for the plant at 10°C. Use a third specimen and determine the number of oxygen bubbles produced by the plant for one minute, for five minutes, and an average at 30°C. Increase the temperature to 40°C and determine the number of oxygen bubbles produced by a fourth specimen in a minute, five minutes, and an average for one minute. Record in the table.

Temperature of water in water bath	*Rate per minute*	*Rate per five minutes*	*Average rate per minute*
10°C			
20°C			
30°C			
40°C			

Construct a separate graph to show the effect of light intensity and temperature on the production of oxygen by elodea plants.

Interpretation. On the basis of your data, what effect does light intensity have on the production of oxygen? ....

....

....

On the basis of your data, what effect does temperature have on the production of oxygen?

....

....

....

C Part 5 EFFECT OF VARIOUS VISIBLE LIGHT WAVELENGTHS ON THE GROWTH OF GREEN PLANTS

Problem. Do seed plants grow better in one wavelength of light, or do they grow equally as well in all wavelengths? Basically, all wavelengths of the visible spectrum can be used in photosynthesis. You can experiment with the relative degree of effectiveness of the various visible light wavelengths on the growth of seed plants by covering green plants with sheets of colored cellophane.

Suggested materials. Healthy bean seedlings (4-in.); others may be substituted; cellophane paper (white or clear, red, blue, green).

Suggested procedure. Use four healthy potted bean or other seedlings. Wrap each of the seedlings with cellophane paper. Be sure to fasten the paper so that light from the outside does not reach the plant. The plant wrapped in clear cellophane acts as a control for the experiment. Place the covered plants in a well-lighted area of the room so that each one receives the same amount of light daily. Water the plants regularly and allow them to grow for one week. Examine the plants, observing the condition of the leaves and stems, their size, and intensity of chlorophyll. Prepare a table and record your results.

Remove a leaf from each of the plants, experimental and control. Use the iodine test to determine the difference in the amount of starch produced by each leaf. Account for any differences you found in starch production.

You might extend your experimentation to include an alga or submerged plant.

Analysis and conclusions. Discuss your results in terms of the ability of the plant to receive energy from the specific color and wavelength of radiation it was supplied in the experiment.

Cellular Respiration 7-1

Purpose To demonstrate that carbon dioxide and energy are produced during respiration.

The activities carried on by a living cell require a continuous supply of energy. This energy is released within the cell as organic molecules, principally glucose, which are oxidized. Carbon dioxide is released as a fuel fragment.

A Part 1 PRODUCTION OF CARBON DIOXIDE BY ELODEA. Although you may associate respiration only with animals, plant cells also require energy for their cell activities and therefore must respire. In this Part you will demonstrate that plants respire by testing for the presence of carbon dioxide.

Materials. Elodea; eight 6-inch test tubes; 2 test-tube racks; brom thymol blue solution; ammonium hydroxide; aquarium water.

Procedure and observations. To determine whether carbon dioxide is present as a result of oxidation, you will need to use an indicator. Brom thymol blue is an accepted indicator for the presence of carbon dioxide. It is blue when the environment is alkaline, but turns yellow in an acid environment, such as when carbon dioxide is present in water and forms carbonic acid.

Dissolve 0.5 gm of brom thymol blue in 50 ml of water. Add a trace of ammonium hydroxide (about a drop per liter) to turn the solution a deep blue. Prepare a second solution containing 50 ml of aquarium water and 20 ml of the brom thymol blue solution. Fill eight 6-in. test tubes to within one inch of the top with the second solution. To four of the tubes, add a vigorously growing sprig of elodea. The remaining tubes will serve as controls.

Put two of the tubes with elodea and two controls in the dark. Put the other tubes in a sunny spot in the room. Observe the tubes carefully. (a) Which of the tubes showed a color change? .... (b) Explain why. ....

A Part 2 RESPIRATION IN GERMINATING SEEDS. In this Part you will determine whether germinating seeds give off carbon dioxide.

Materials. Box of seeds; 4 gas bottles; 4 one-hole stoppers to fit gas bottles; 2 pieces of bent glass tubing; 5% formalin solution; 10% formalin solution; brom thymol blue solution.

Procedure and observations. To disinfect the seeds you are to use, soak them about 20 minutes in a 5% formalin solution. Then wash the seeds with cold water and soak them overnight.

Fill a gas bottle about one-third full of the seeds. Fill a second gas bottle about one-third full of brom thymol blue. Use a U-tube to connect the two gas bottles as shown in Fig. 7-1-a. As a control, set up a similar apparatus, substituting killed seeds for the living ones. (Seeds may be killed by soaking them in a 10% formalin solution for an hour.) Observe carefully. (a) What color change, if any, occurs? ....

(b) Explain the results in the experiment. ....

....

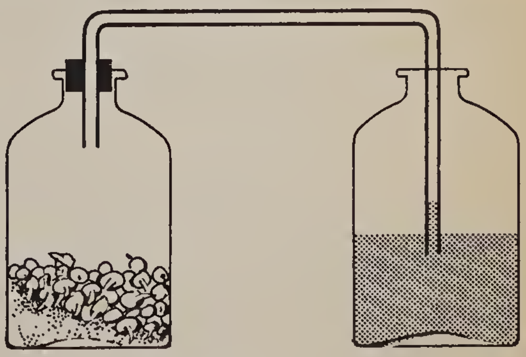


Fig. 7-1-a Apparatus for testing CO_2 production in seeds

B Part 3 ENERGY RELEASE DURING RESPIRATION. During respiration, chemical energy present in the bonds of glucose molecules is released as the glucose is oxidized by enzyme action. Some of this energy is used to support cell activities. Much is given off as heat. The amount of energy released depends on the degree to which the glucose molecules are broken down. The more complete the respiration process, the greater the release of energy.

Fermentation is a type of respiration in which glucose is oxidized and carbon dioxide and ethyl alcohol are formed. Only five percent of the energy in glucose is released. The other 95 percent remains locked in the bonds of ethyl alcohol.

In this Part you will use yeast cells to ferment sugar in apple cider and determine that energy is given off as heat in the process.

Materials. Fresh apple cider; dry yeast; 2 gas bottles; thermometers; rubber tubing; 2 two-hole stoppers to fit Thermos bottles; 2 one-hole stoppers to fit gas bottles; 4 pieces of glass tubing; 2 Thermos bottles; brom thymol blue solution or limewater.

Procedure and observations. Fill a Thermos bottle two-thirds full of fresh apple cider and add 2 gm of dried yeast. Insert a piece of glass tubing as shown in Fig. 7-1-b into one of the holes of a two-hole stopper. Then insert a thermometer into the second hole so that it will extend into the apple cider when the stopper is placed in the Thermos bottle. Fill a gas bottle one-half full of limewater or brom thymol blue. Now put the stopper in the Thermos bottle and insert the long end of the glass tubing into the one-hole stopper of the gas bottle, as shown in Fig. 7-1-b. Identify this Thermos bottle as the experimental bottle. As a control set up a similar apparatus omitting the yeast. Observe the experiment carefully, recording in the table provided, the temperature of the apple cider of both the experimental and control bottles at the beginning and at twenty-four hour intervals for seventy-two hours.

(a) Was energy produced in one, both, or neither Thermos bottle? ....

(b) Account for your answer. ....

....

Examine the two gas bottles. (c) Describe the content of both bottles. ....

.... (d) Account for any change you observed in the gas bottles.

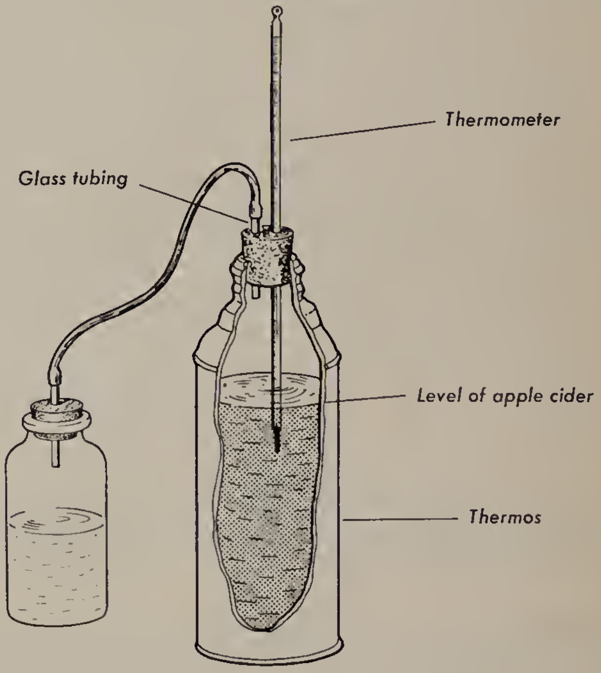


Fig. 7-1-b Apparatus for demonstration of energy release

Thermos Bottle	Temperature			
	Start	24 hours	48 hours	72 hours
Control				
Experimental				

.... (e) What product other than carbon dioxide is produced during fermentation? ....

(f) How can you prove this? .... (g) What kind of respiration are the yeast cells carrying on? .... (h) Explain your answer. ....

....

Interpretation. On the basis of your demonstration, explain why fermentation occurred in one Thermos, but not in the other. ....

.... Even in the presence of free oxygen, yeast cells continue to produce alcohol and are unable to complete a process of aerobic respiration. See if you can find an explanation for this. ....

....

Cell Growth and Reproduction

8-1

Purpose To observe changes in chromosomes and other nuclear materials during mitosis and diagram chromosome changes in meiosis and fertilization.

With the exception of the reproductive cells, all the cells composing the body of an organism have identical chromosomal makeup. How is this accomplished through the many thousands of cell divisions that occur during the growth of the organism? The genetic code, which makes a cat a cat or a pine tree a pine tree, operates through genes borne on chromosomes. Genetic continuity is preserved by replication and equal distribution of genetic materials by the process known as mitosis. During the process called meiosis, each cell receives only half of the genetic materials, thus providing the mechanism for equal genetic contribution of two parents during sexual reproduction.

A Part 1 STUDY OF MITOSIS USING PREPARED SLIDES. Mitosis is divided into a series of four mitotic stages: prophase, metaphase, anaphase, and telophase. When the cell is preparing for mitotic division, it is in the interphase. You can study the various stages of mitosis by examining slides prepared with special stains developed to show chromosomes and nuclear materials difficult to see in living cells.

Materials. Prepared slides of mitosis (onion root tip and whitefish blastula); microscope.

Procedure and observations. Section 1 *Study of mitotic stages in onion root tips.* Scan the onion root tip under low power of your microscope and locate the mitotic stages, which are found in the region between the tip of the root and the region where the cells are beginning to elongate. Turn to high power to study the individual cells more closely. Locate as many stages of mitotic division as you can find. These stages are distinguished by the density of the chromosomes, by the presence or absence of the nuclear membrane, by the presence or absence of the nucleolus, by the development of a spindle, by chromosome attachment by centomeres, by the migration of the chromosomes along the spindle fibers to the poles, and by the reorganization of the nucleus and division of the cell. These are the features that should appear in your drawings. Study the cells carefully and hunt for the interphase, prophase, metaphase, anaphase, and telophase stages of mitosis. Draw these in the spaces provided and summarize the changes that occur during each stage at the right of the drawing.

(a) What role does the spindle play in a dividing cell? ....

(b) Where does the cellulose wall form in the mother cell? .... (c) What is the function of the wall? ....

Section 2 *Study of mitotic stages in whitefish blastula.* Now examine a prepared slide showing mitosis in the whitefish blastula. Locate as many stages of mitosis as you can find. Look for a cell in which the chromatids are visible. Study the poles of the spindle and compare them with those of the onion root tip. (d) How do they differ from those of the onion? .... Locate a cell in which daughter cells are being separated. (e) Compare this separation with the plant cell wall. ....

....

(f) What structures can you find in the dividing animal cell that are not present in the plant cell? .... (g) What structures

Mitotic figures	Significant changes
Interphase	
Prophase	
Metaphase	
Anaphase	
Telophase	

Stages in mitosis

can you find in both dividing plant and animal cells? ....

....

A Part 2 MEIOSIS AND FERTILIZATION. In addition to mitosis, the cells of the reproductive organs also undergo meiosis, which results in the reduction in chromosome number from the diploid to the haploid. This process is necessary when gametes are formed by the body cells of the reproductive organs. Thus, when the two haploid cells (egg and sperm) are united during the process of fertilization, the zygote formed contains the diploid number of chromosomes.

Materials. Colored pencils.

Procedure and observations. Simplified diagrams of egg and sperm formation are shown in Fig. 8-1-a. The first stage in egg formation (oogenesis), shown on the right, is the primary oocyte. Draw four pairs of chromosomes in this cell, making one pair red, one pair blue, the

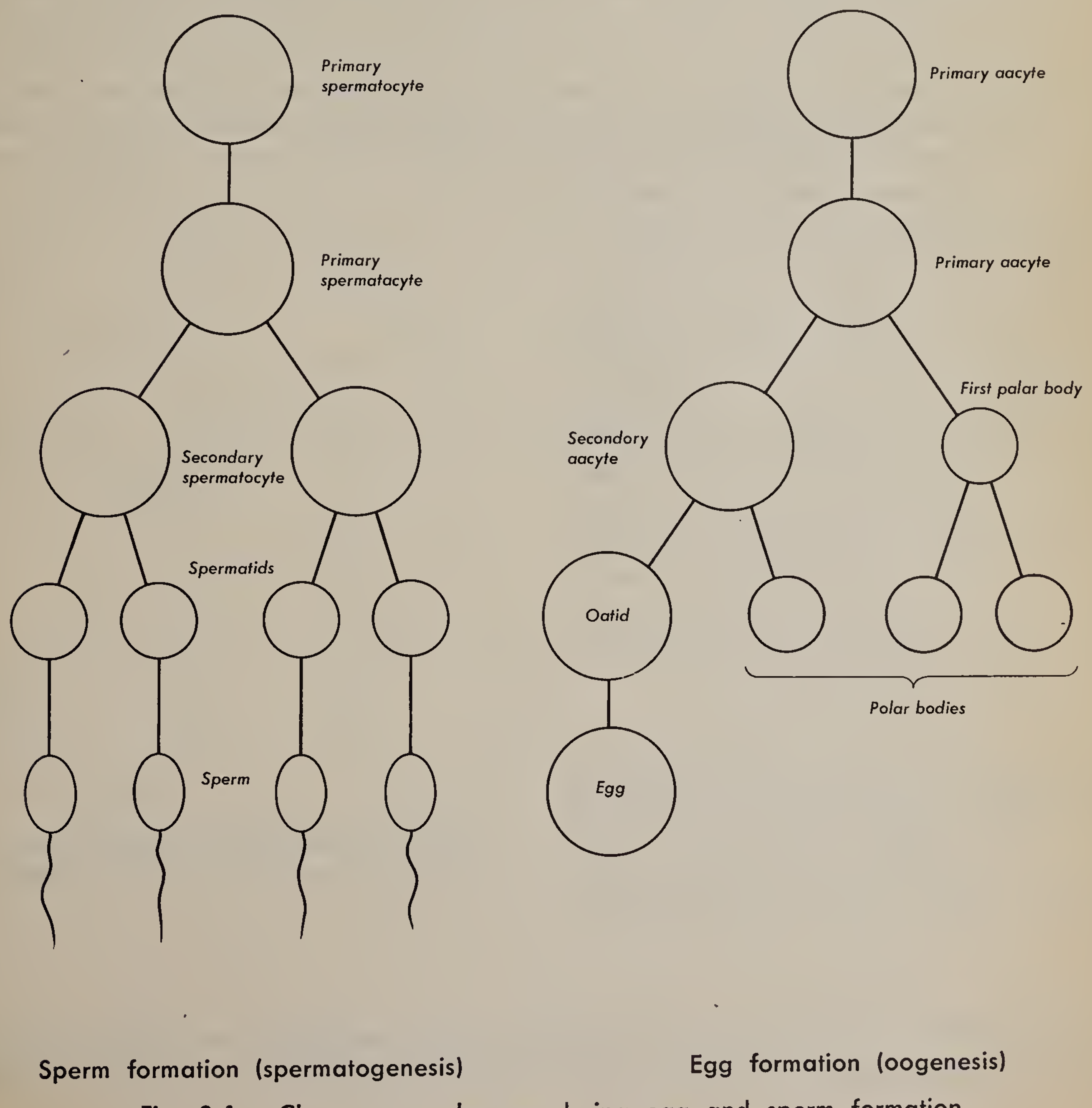

Primary spermatocyte
Primary spermatacyte
Secondary spermatocyte
Spermatids
Sperm
Primary aacyte
Primary aacyte
First palar body
Secondory aacyte
Oatid
Polar bodies
Egg
Sperm formation (spermatogenesis)
Egg formation (oogenesis)

Fig. 8-1-a Chromosome changes during egg and sperm formation

third pair green, and the fourth pair yellow. Mark one chromosome of each pair *A* and the other *B*. As the primary oocyte develops and becomes the second cell shown, all of the chromosomes replicate, but do not separate. This results in a tetrad, or group of four chromatids. Show these chromatids, using the same colors as those you used for the original chromosomes and indicating them as *A* and *B* to correspond with those in the first cell. The chromatids form pairs and separate from the tetrad and move toward the opposite poles. Following this migration, the primary oocyte divides, forming a secondary oocyte and the first polar body. These cells contain one of the original pair of homologous chromosomes, each composed of joined chromatids. This is the stage in which real reduction division ocurs. Draw these chromosomes composed of joined chromatids in the secondary oocyte and first polar body. Identify these as *A* and *B* to indicate their separation.

The chromatids separate during the division of the secondary oocyte to form the ootid and a second polar body. The ootid and second polar body then contain the haploid (*n*) chromosome number. The first polar body divides to form two additional second polar bodies, each containing the haploid (*n*) chromosome number. The ootid matures into an egg. Fill in the chromosomes in the egg cell and the three polar bodies, which have no function in reproduction and gradually disappear.

Sperm formation (spermatogenesis) is identical, except that the primary spermatocyte divides to form two secondary spermatocytes. Thus, no polar bodies are formed and four sperm result. Show the chromosome changes during sperm formation just as you did in egg formation.

Section 2 *Diagram of chromosome changes during fertilization and formation of the zygote.* The egg and sperm produced in oogenesis and spermatogenesis are shown in Fig. 8-1-b. Each of these cells contain the haploid (*n*) number of chromosomes. Show these chromosomes, using the color scheme from Section 1. Indicate each as *A* or *B*. The line joining the cells indicates union of the two during fertilization. When the zygote is formed, both cells contribute a homologue of a chromosome pair and the diploid (*2n*) number is re-established. Fill in the chromosomes in the zygote. Immediately after fertilization, the zygote divides, forming two cells. This division follows mitosis, during which each chromosome replicates. Thus, each of the new cells receives the *2n* number of chromosomes. Show the chromosome content of the two cells resulting from division of the zygote in Fig. 8-1-b.

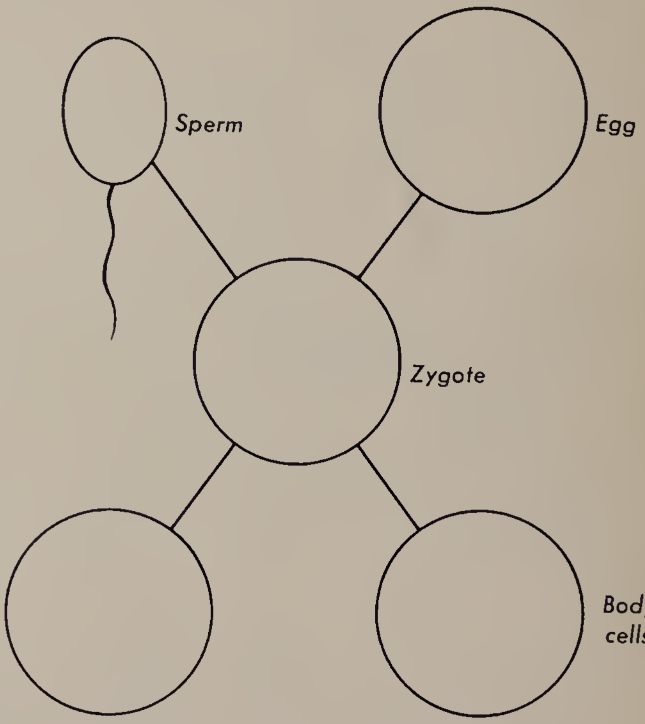


Fig. 8-1-b Chromosome changes during fertilization and division of the zygote

B Part 3 **PREPARING SMEARS OF ONION ROOT TIPS.** Now that you have become familiar with the stages in mitosis from previously prepared slides, you are ready to prepare some slides of your own showing mitosis.

Materials. Fresh onion; microscope; slides; cover glasses; single-edge razor blade; beaker or tumbler; applicator sticks or toothpicks; watch glass; filter paper; Bunsen burner; acetocarmine dye.

Procedure and observations. The live onion root tip is a good source for your mitosis study. Select a fresh, firm onion and cut off the dried roots close to the base of the bulb. Fill a beaker or tumbler (any glass container will do) two-thirds full of water. Insert three applicator sticks or toothpicks equidistant from one another and at right angles to the onion, so that the sticks support the onion in the water. Suspend the onion in the water as shown in Fig. 8-1-c and set the beaker in a dark place. Observe the onion daily until new white root tips appear and grow to a length of ½-1 in. When these have formed, fill a watch glass with acetocarmine stain. Remove the onion from the water and, using a sharp single-edge razor blade, slice off the lower, denser half of the onion root tip into the watch glass. Heat the tips in the stain over a moderate flame for three to five minutes. *Do not permit the stain to boil.* Remove the tip from the watch glass and place it on a clean slide with a drop of fresh acetocarmine stain. Examine the root and cut off the portion of the root tip (about 1/16 in.) which is most deeply stained. Macerate this portion with your razor blade on the slide until the pieces are less than the size of a pinhead. Mount with a cover glass. Using a pencil eraser or the ball of your thumb, press firmly on the cover glass and mash the material under it to a thin layer. *Do not let the cover glass slip.* Use filter paper or paper toweling to blot the excess stain from the slide.

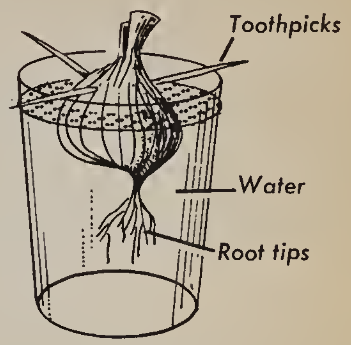


Fig. 8-1-c Technique for growing onion root tips

Examine your slide under the low power of your microscope and locate cells that have dark-stained, threadlike structures, the chromosomes. Use the high power of your microscope to study the individual cells more closely. Study the slides that other members of your class have made. Locate as many stages of mitotic divisions as you can find. Compare these with the prepared slides. There are sixteen chromosomes in the cell during replication. (a) Can you locate them in the prophase stage? .... (b) Explain why or why not. ....

....

Interpretation. Why was it necessary to use growing tissue in order to see the chromosome structures in the cell? ....

....

Genetic Facts and Fallacies 9-1

Purpose To determine the extent to which some basic genetic principles and concepts are understood.

Have you ever heard anyone say that the male is the stronger of the two sexes and that he controls the heredity of the child? Or have you heard anyone suggest that an expectant mother attend concerts if she wants her unborn child to become a musician? Through our knowledge of genetics, we have learned that the mother does not influence her unborn child, nor does the father alone control its heredity. In this survey you will determine the extent to which members of your family and your friends believe various statements concerning inheritance.

A Part 1 CONDUCTING THE SURVEY. The following statements relate to various genetic principles, many of which are associated with common false ideas and superstitions. Certain

	True	*False*	*Total*
1. Certain acquired characteristics, such as mechanical or mathematical skill, may be inherited.			
2. Identical twins are always of the same sex.			
3. Fraternal twins are more closely related to each other than to other children in a family.			
4. The father determines the sex of a child.			
5. Each parent contributes half of a child's genetic makeup.			
6. Certain thoughts or experiences of a mother may mark or alter the hereditary makeup of an unborn child.			
7. Color-blindness is more common in males than in females.			
8. A person may transmit characteristics to offspring which he, himself, does not show.			
9. Certain hereditary characteristics are influenced by the blood.			
10. Identical twins are more closely related than fraternal twins.			
11. Certain inherited traits may be altered by the stars, moon, or planets early in development.			
12. Males are biologically stronger than females.			
13. The tendency to produce twins may run in families.			
14. A craving for a food such as strawberries may cause a birthmark on an unborn child.			
15. Many of a person's inherited traits do not appear.			
16. The parent with the stronger will contributes more to a child's inheritance than the other parent.			
17. If a person loses a limb in an accident, it is likely that he or she will have a child with a missing limb.			
18. The attitude of parents toward each other influences the emotional makeup of an unborn child.			
19. Children born to older parents usually lack the vitality of those born to younger parents.			
20. The total number of male births exceeds female births each year.			

of the statements are true. Others are false. The purpose of the survey is not to test you, but to provide a survey for you to use.

Materials. No materials or apparatus required.

Procedure and observations. Select ten or more people of various ages and read each of the 20 statements. Ask each person if he believes the statements to be true or false. Tabulate the answers given after each statement and show the total number of subjects who responded to each statement. Be careful not to indicate the answer in reading the statement.

Correct answers to all survey statements are given below. Indicate the correct and incorrect answers on your survey. Then fill in the following table, giving results for the entire class.

	No. correct answers	*No. incorrect answers*	*Total responses*	*% correct answers*	*% incorrect answers*
1. False					
2. True					
3. False					
4. True					
5. True					
6. False					
7. True					
8. True					
9. False					
10. True					
11. False					
12. False					
13. True					
14. False					
15. True					
16. False					
17. False					
18. False					
19. False					
20. True					

Using a sheet of graph paper, make a bar graph showing the percent of correct and incorrect responses to each of the questions in the survey. Use different colors to distinguish correct and incorrect responses. Number the 20 survey questions along the bottom of the graph and use the vertical graduations to indicate the percentage of correct and incorrect responses.

Purpose To study Mendel's laws of unit characters, dominance, segregation, and independent assortment.

The laws that Mendel formulated from his experiments with garden peas stand today, practically unchanged. His conclusions concerning the inheritance of traits were based on the study of many generations of garden peas. Since you cannot study several generations of plants or animals during your biology course, we have provided several situations in which you can study Mendelian laws of inheritance and determine the expected ratios of offspring from parents whose genotypes are provided.

A Part 1 MONOHYBRID CROSS. The seed coat was one of the seven pairs of contrasting traits that Mendel studied in garden peas. He found that some seeds had smooth coats and others had wrinkled coats. He crossed plants having these two types of seeds to determine how the seed coat trait is inherited.

Materials. No materials or apparatus required.

Procedure and observations. The parent plants in the cross Mendel used are shown in Fig. 9-2-a. The plant that is homozygous for smooth seeds is designated as *SS*, while *ss* indicates the genes in the plant that is homozygous for wrinkled seeds.

(a) If the male parent is homozygous for smooth seeds, what kind of gene will be present in each sperm produced? ....

(b) Similarly, if the female parent is homozygous for wrinkled seeds, what kind of gene will the eggs contain? ....

Diagram this cross in the Punnett square marked *F₁*. (c) What is the genotype of the plants in the F_1 generation? ....

(d) What is the phenotype of the plants in the F_1 generation? ....

(e) How does this cross illustrate the law of unit characters? ....

....

(f) of dominance? ....

....

(g) In what way do the offspring of the F_1 generation differ from the parents? ....

....

Mendel then crossed two heterozygous smooth-seed plants (*Ss*) to produce the F_2 generation. Diagram this cross in the Punnett square marked *F₂*.

(h) What is the phenotype ratio of the offspring? ....

P	♂ Smooth seeds SS	♀ Wrinkled seeds ss
Female GENES Male		
F₁		

	♂ Hybrid smooth Ss	♀ Hybrid smooth Ss
Female GENES Male		
F₂		

Fig. 9-2-a A cross involving one pair of genes with dominance

(i) What two kinds of smooth-seed plants are present in the F_2 generation? ....

....

(j) Which of Mendel's laws are demonstrated in this cross? ....

....

A **Part 2 DIHYBRID CROSS.** In Part 1 you diagrammed the inheritance of a single trait. In this Part you will study crosses in which two traits are involved.

Materials. No apparatus or materials required.

Procedure and observations. In the P generation shown in Fig. 9-2-b, the male parent is black and rough-coated and is homozygous for both of these characters (*BBRR*). Black and rough are dominant in guinea pigs. The female parent is a homozygous white, smooth-coated guinea pig (*bbrr*). Both of these characters are recessive. (a) What genes are in the sperm? ....

(b) What genes are in the eggs? ....

....

(c) What is the genotype of the F_1 generation?

....

(d) What is the phenotype of the F_1 generation?

....

Two of the offspring of the F_1 generation are used as parents to produce the F_2 generation in Fig. 9-2-b. Diagram the possible offspring from such a cross in the Punnett square and list the ratio of the four phenotypes below the Punnett square. (e) How does this cross illustrate the law of independent assortment? ....

....

(f) If the genes for coat color and those for coat texture were carried on the same chromosome, would this law apply? .... Explain your answer. ....

....

....

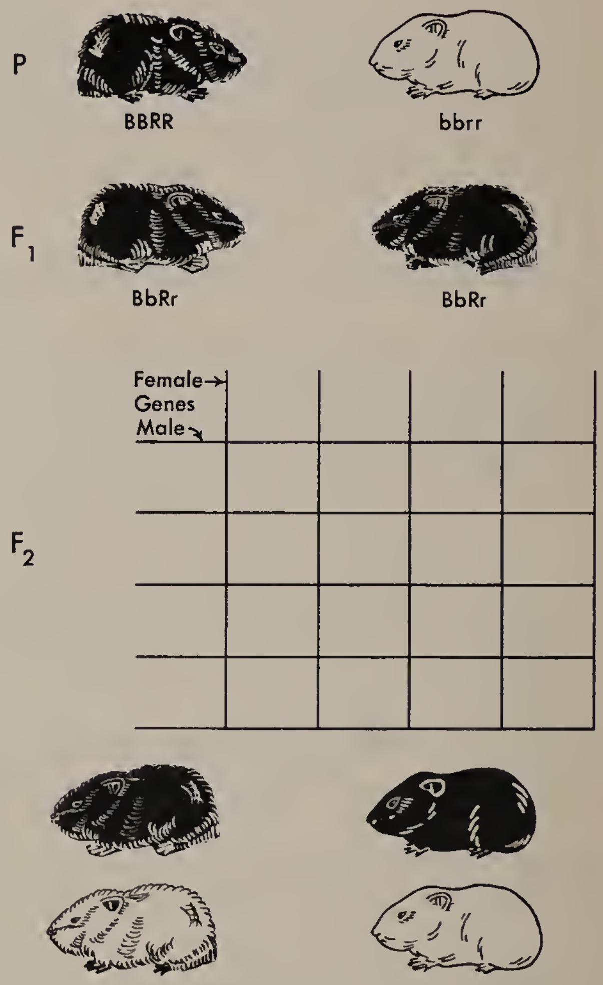


Fig. 9-2-b A cross involving two pairs of genes with dominance

Ratios

Black rough ..... White rough .....

Black smooth ..... White smooth .....

B **Part 3 STUDY OF INHERITED TRAITS IN CORN SEED.** There are two very striking contrasting inherited characteristics found in corn seeds. One is the color of the outer layer of the endosperm (purple or nonpurple) and the other is the characteristic of the seed coat (smooth or wrinkled). When pure strains of corn producing purple seeds are crossed with pure strains of corn producing nonpurple seeds, all of the F_1 offspring have purple seeds. Furthermore, when pure strains of corn producing smooth seeds are crossed with pure strains producing wrinkled seeds, all the F_1 offspring have smooth seeds.

Materials. Genetic corn showing a 1:1 ratio of colored-colorless seeds (purple-yellow); genetic corn showing a 3:1 ratio of colored-colorless seeds (purple and yellow); genetic corn showing a 9:3:3:1 ratio purple-smooth-yellow-shrunken seeds; straight pins.

Procedure and observations. Section 1 *Monohybrid cross.* Some of the members of the class will be given ears of corn that were produced by crossing two hybrid purple parent strains; others will receive ears that were produced by crossing a hybrid purple parent strain and a nonpurple parent strain. You will not be told what kind of cross produced the ear of corn you have. You must determine this for yourself by counting the number of kernels of each color (purple or yellow) you find on the ear. Put a pin in the end of the row of kernels when you begin the counting. Count the characteristics of each kernel in a row until you return to the point marked by the pin. Record your count in the table provided. *Do not pick the kernels from the ears.*

Kind of Seeds	*Number in Each Row*												
	1	2	3	4	5	6	7	8	9	10	11	12	*Total*
Purple													
Nonpurple (Yellow)													

Determine the total number of purple and nonpurple seeds you have on your ear of corn. Record the total in the table provided. (a) What percent of the seeds were purple? ....

(b) nonpurple? .... (c) What is the ratio of purple to nonpurple seeds?

.... (d) Is there any evidence of purple color in the nonpurple seeds?

.... (e) Explain your answer. ....

.... (f) The purple seeds on both kinds of ears of corn that members of the class have counted resemble one another in color, what evidence did you find that they may differ genetically? ....

....

(g) List the Mendelian laws illustrated in this Part. ....

....

Interpretation. On the basis of your results, reconstruct the genetic makeup of the parents of the two kinds of ears of corn. ....

....

Section 2 *A dihybrid cross.* You will not be told what kind of plants were crossed to obtain the ear of corn you have. Your counting of the characteristics (purple, nonpurple, smooth, and wrinkled) should help you determine the possible genotypes of the parents. Use the same procedure as was used in Section 1 of this Part to count and classify the seeds. Record your count in the table provided.

Kind of Seeds	*Number in Each Row*												
	1	2	3	4	5	6	7	8	9	10	11	12	*Total*
Purple-smooth													
Purple wrinkled													
Nonpurple smooth													
Nonpurple wrinkled													

(h) How many different phenotypes did you find? ....

(i) Name these types. ....

(j) Which type appeared most frequently? ....

(k) less frequently? .... (l) Were the purple wrinkled seeds as common as the nonpurple smooth ones? .... (m) Using the symbols *P* for purple and *p* for nonpurple and S for smooth and *s* for wrinkled, give the genotypes of the F_1 generation that produced the ear of corn you examined. ....
The two parents that produced the F_1 generation were pure strains. (n) Give the genotypes of the parents of the F_1 generation. ....

(o) Which Mendelian law is represented in this section? ....

....

Interpretation. On the basis of your results, explain how genetic principles operate according to predictable rules. ....

....

C Part 4 STUDY OF INHERITANCE OF SIZE IN PEAS

Problem. How is height inherited in pea plants?

Gregor Mendel determined that the gene for tallness in pea plants was dominant over the gene for dwarfness. You can duplicate his work by using tall and short peas of known genetic strain as parent plants and producing an F_1 and F_2 generation under controlled experimental conditions.

Suggested materials. Twenty-four little Marvel or Progress #9 strain seeds homozygous for dwarf plants; 24 Alderman variety homozygous for tall pea plants; flats for planting seeds; 4-5 inch flowerpots; potting soil; supports for tall plants; forceps; string.

Suggested procedure. Obtain seeds for both dwarf and tall pea plants from your teacher. Plant the two types of seeds three inches apart in germinating flats so that they are covered by one inch of soil. Put the flats near a window. You may put them outside if the temperature is not too low. Water as needed. After five to ten days, select 12 of the most vigorous plants of each type and plant them in 4-5 inch flowerpots. (You can determine which plants are dwarf ones after the five- to ten-day interval.) Provide the tall plants with some type of support.

Cross-pollinations will have to be done before the flowers open. If you do not cross-pollinate the flowers before they open, the anthers will shed their pollen onto the stigma and self-pollination will occur. Remove the brightly colored portion of the flower to expose the stamens and pistil. The stamen consists of a slender stalk with a more or less cylindrical anther at its apex. Pollen grains, which are responsible for the production of male reproductive cells, or sperm, are contained in the anther. The pistil is found in the center of the flower. This is the female part of the flower; it contains the ovary, within which seeds are formed following fertilization by the sperm.

Use a pair of forceps to remove all the stamens from the plants. Then dust the pollen from an anther onto the tip of the pistil. Be sure to record the crosses you make and to clean the forceps each time you start to cross-pollinate a new set of flowers so as to avoid contamination of the stigmas with other pollens you have used.

Allow the plants to grow until pods have developed. After the pods have ripened, remove them carefully, being sure to label each one as to the type of cross producing it. Put all the pods from similar crosses into one container.

To determine what kind of plant each seed will produce, plant each seed as you did previously. Keep a record of where each seed is placed. After five to ten days record your data and determine whether the plants are tall or dwarf. These plants represent the F_1 generation. To produce an F_2 generation, cross F_1 plants in the same way that you crossed the parent plants. Plant the resulting seeds to determine the height ratio in the F_2 generation.

Analysis and conclusions. Summarize your data in Punnett squares or in table form. Your study should result in an understanding of how Mendel's laws of unit characters, dominance, and segregation apply to the inheritance of height in garden peas.

Chromosomes and Genes 10-1

Purpose To study the chance distribution of genes in eggs and sperm resulting from meiosis during oogenesis and spermatogenesis, and to study the salivary glands of the fruit fly.

The study of genetics reveals that hereditary traits are determined by specific areas of a chromosome called genes. Genes are arranged in linear order on the chromosome and occur in pairs, as do the chromosomes. Chance distribution of chromosomes during meiosis and their recombination during fertilization account for the many variations that occur in offspring.

B Part 1 DEMONSTRATION OF THE CHANCE COMBINATION OF GENES DURING FERTILIZATION. In this Part you will study the random, or chance, combination of two kinds of beans. There are only three possible combinations these beans can make. This represents what actually occurs during fertilization when a gene pair lying on two homologous chromosomes recombines after being separated during meiosis.

Materials. Eight boxes; 1,600 red beans; 1,600 white beans.

Procedure and observations. The class should be divided into four sections for this activity. Each section will have two boxes of beans. Each box contains 400 red beans and 400 white beans. Label one box to represent the genes contributed by the sperm and the other to represent genes contributed by the egg.

Since the egg and the sperm each contribute one gene of a single pair, choose a bean from each box in order to have a gene pair. Lay the gene pairs in rows: red-red, red-white, and white-white. Select your beans in a series of rounds which will be timed by your teacher. After each round, count the beans in each row and record in the table provided.

Round	*Number of Pairs of Beans In:*		
	Row 1 *red-red*	*Row 2* *red-white*	*Row 3* *white-white*
1			
2			
3			
4			

On the basis of the data you collected, it is possible to calculate the ratios for each type of gene pair. Add the totals of your three rows and divide the sum by four. (The *four* represents the reduction that occurs in the formation of the egg and sperm during meiosis.) Then divide the quotient into the total for each row. The number you obtain expresses a ratio. A sample of this calculation is given in Fig. 10-1-a.

$37 + 86 + 41 = 164$

$164 \div 4 = 41$

$37 \div 41 = .9$

$86 \div 41 = 2.1$

$41 \div 41 = 1.0$

Ratio .9 red-red;
2.1 red-white;
1.0 white-white

Fig. 10-1-a Calculation of ratio

(a) What ratio did you obtain? ……………………………………

…………………………… (b) Why is it necessary to have so many beans in each box? ……………………………………

(c) Explain why the number of each color is constant in all boxes.

……………………………………

(d) Why was it necessary to select so many pairs? ....

.... (e) What were your chances of selecting the same color of beans in a gene pair? .... (f) a different color?

.... (g) Explain why it was important to use two different colors? ....

(h) What genetic principles are demonstrated by your bean selection? ....

Interpretation. How does chance selection of genes provide the basis for variations in organisms? ....

....

....

B Part 2 STUDY OF SALIVARY CHROMOSOMES IN *DROSOPHILA*. The salivary glands of the fruit fly are made up of giant-sized cells with unusually large chromosomes. Because of their size, these chromosomes have been used extensively in genetic studies. In this Part you will be given the technique for dissecting, staining, and studying the salivary chromosomes of the fruit fly.

Materials. Culture of fruit fly larvae; microscope; slides; cover glasses; dissecting needle; forceps; paper towels or filter paper; carmine powder; 45% acetic acid; 0.7% sodium chloride solution.

Procedure and observations. Study the fruit fly larvae in the container on your desk and locate the largest one. Insert a pair of forceps and remove this larva. Put it on a slide and study it closely. Find the anterior end. It is pointed and you can see the black mouth parts as they move back and forth.

Now put a drop of 0.7% sodium chloride solution on the larva. Locate the head end and pierce it with a dissecting needle. The larva wiggles, and you will probably need to make several attempts before you finally pierce the head. Then pierce the posterior end and hold it down, as shown in Fig. 10-1-b. Slowly stretch the larva until the mouthparts tear off. You may need to sacrifice several larvae before you are successful.

After you have removed the mouthparts, mount them on a clean slide and examine them under low power of the microscope. Compare your material with the sketch in Fig. 10-1-b. Now free the glands from the digestive tube and push them to one side. Remove any other parts of the larva.

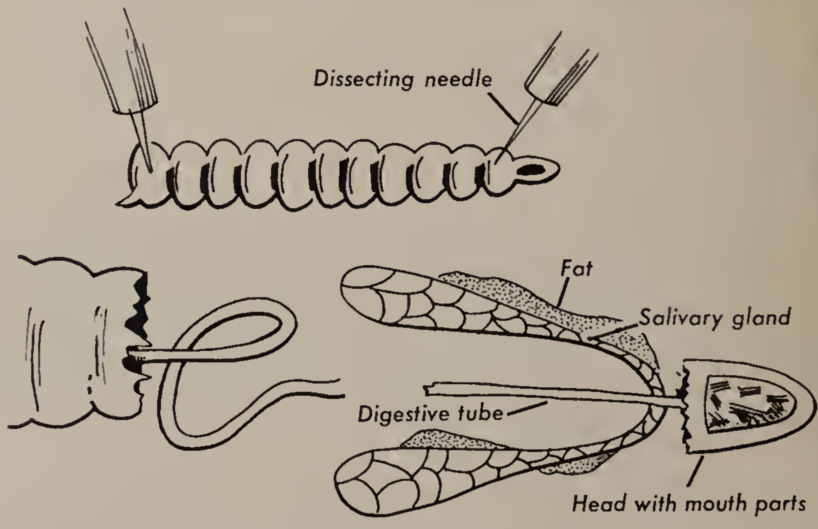


Fig. 10-1-b Technique for removing salivary glands of fruit flies

Use filter paper or a small piece of paper toweling and remove the excess salt solution around the glands. *Avoid touching the glands.*

Staining the salivary glands will enable you to see the chromosomes better. Acetocarmine is the preferred stain because it differentiates the nucleus. The procedure for preparing acetocarmine is as follows: To a boiling 45% acetic acid solution add carmine powder until the solution is saturated. Then filter the solution.

Place one drop of acetocarmine stain on the salivary glands. Observe the action of the stain under the low power of the microscope. Allow the stain to stand for five minutes. Put a piece of paper toweling around your thumb to soak up the stain. Now carefully place a *plastic* cover glass on top of the stained glands and press it down with your thumb. Examine the slide under the microscope. (a) What is the condition of the gland? ....

.... (b) Can you identify the nucleus in most of the cells? ....

Examine the nuclei and locate the coiled giant chromosomes. Focus very carefully and slowly on these chromosomes. (c) Can you see any structures in the chromosomes? .... Make a sketch in the space provided of several cells and show all the structures you can see. Identify and label: **nucleolus, nucleus,** and **chromosome bands.**

Removing the chromosomes from the nucleus is a very difficult procedure. Remove the slide from the microscope stage and tap the cover glass *very hard* 10-20 times with the handle of the dissecting needle.

After you have tapped the cover glass, examine the slide under the microscope. You should find a nucleus which split and released the giant chromosomes. If you were not successful with the tapping, repeat the process and examine again. If you find a chromosome or a piece of one by itself, focus on it and look for its structures. (d) Describe the chromosome. ....

....

Cells of salivary gland

(e) Can you locate the genes? ....

Interpretation. From your study of salivary chromosomes in *Drosophila,* discuss the characteristics of these chromosomes that make them ideal subjects for genetic study. ....

....

....

....

....

Purpose To learn the technique of culturing and crossing fruit flies; to study their inherited characteristics; to demonstrate Mendelian laws by mating special types of fruit flies.

Drosophila melanogaster, the common fruit fly, has been used for research in genetics for many years. The fruit fly is ideal for research because its reproductive cycle is short, large numbers of offspring are produced, it has many contrasting traits which can be easily observed, and it has only four pairs of chromosomes.

B Part 1 GENETIC CROSSES IN FRUIT FLIES. You can make interesting crosses by using wild-type flies, which are those typically found in nature. These flies can easily be collected by leaving a culture bottle open out of doors when the flies are in season.

Materials. Wild-type fruit flies; hand lens; 25 culture jars (8 oz, 2 oz, or half-pint milk bottle); two 4-oz jars; cotton plugs or caps for jars; cork or stopper to fit one of the jars; camel's hair brush; nail; string; filter paper or paper toweling; autoclave or pressure cooker; refrigerator; ether; ripe banana; yeast cake; fermenting fruit; nutrient agar; cornmeal; corn syrup (molasses); distilled water; Petri dishes.

Procedure and observations. Section 1 *Preparation of media.* Prepare a yeast suspension as a temporary medium for collecting the flies by dissolving one-fourth cake of yeast in 100 ml of water. Then dip a piece of ripe banana into the yeast suspension. Put this piece of banana and a strip of filter paper into a clean glass bottle.

In order to have materials available for long-range study of heredity in fruit flies, you should prepare a culture medium containing agar. Various media are suitable for this purpose. However, one often suggested by geneticists may be prepared as follows: Add 15 gm of agar to 570 ml of water and bring to a boil. Slowly add 100 gm of cornmeal to the boiling solution, stirring continuously. Add 135 gm of corn syrup (Karo) or molasses and allow to boil for five minutes.

Pour this medium into sterilized jars. Use two inches of medium in 8-oz culture jars and one-half inch in 2-oz jars. Plug the jars with cotton or cover with caps. The quantity you prepared will fill 25 culture jars. This will be sufficient for your work in this Investigation.

Sterilize the prepared culture jars in a pressure cooker at 15 pounds for 15 minutes. Remove the jars from the pressure cooker, allow to cool, and insert strips of paper toweling or filter paper cut the length of the jar and folded double into the medium. This provides the flies with a place on which they may crawl to pupate. Slant the jars and allow the agar to gel. See Fig. 10-2-a for illustration of the culture jar and slanting technique.

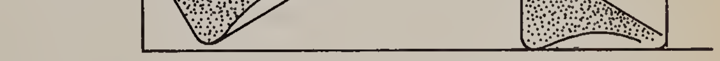


Fig. 10-2-a Culture jars

Refrigerate the culture medium until needed for growing the flies. Just before using, add a piece of active dried yeast to several drops of water and add a few drops of this mixture to the surface of the culture. This will provide the larvae with food.

Section 2 *Collecting and culturing the fruit flies.* If the flies are in season, place an open jar containing the temporary medium on a window sill, and the flies will be attracted to it.

Transfer some of the flies you collected to the culture jars with the long-range medium. These flies will lay eggs on the medium, and in four or five days small larvae may be seen. In about eight days, the pupae will appear as small, yellow-brown cases, about the size of small seeds. The new generation of adult flies will begin to emerge from the pupa cases in about a week. The whole life cycle is completed in 12-16 days. Rearing jars must be kept at a temperature of about 75°F and should not be exposed to bright light. Cultures can be kept alive by adding a few drops of yeast suspension every week. The cultures will grow in the winter.

Section 3 *Etherizing and sorting fruit flies.* In order to transfer, count, or examine the flies, you must etherize them. You can make an etherizing jar from a jar of the type you are using for your cultures. Select a nail an inch longer than the cork or stopper that fits the jar and insert it through the stopper. Wrap thick layers of absorbent cotton around the nail and tie it with a string. Put a few drops of ether on the cotton when it is to be used. (CAUTION: *Ether is highly flammable. Do not use in a room with an open flame.*) When you are ready to transfer the flies, tap the stock jar gently on the table to dislodge the flies from near the mouth of the jar. After the flies have fallen to the bottom, quickly remove the plug of the stock jar, place the mouth of the etherizing jar to the mouth of the stock jar and hold the jars firmly together, as shown in Fig. 10-2-b. Now invert the jars so that the stock jar is on top. Tap the stock jar slightly, so that the flies will move into the etherizing jar. An electric light placed near the etherizing jar will attract the flies. Now separate the jars and stopper both, as shown in Fig. 10-2-b. The flies will be etherized in about 10 seconds. After the flies have fallen to the bottom of the etherizing bottle, and stop moving, remove the stopper and tap the jar to pour the flies on to a clean, white card. If the wings of the fly stick out at an angle, it is dead. Brush it to one side.

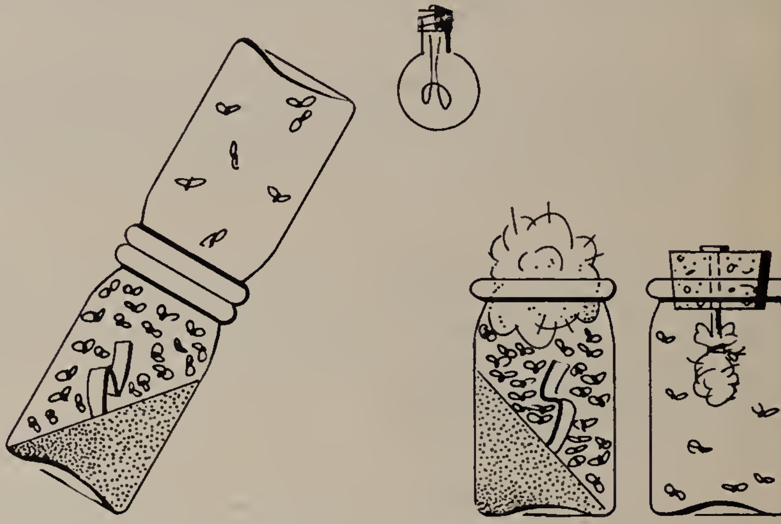


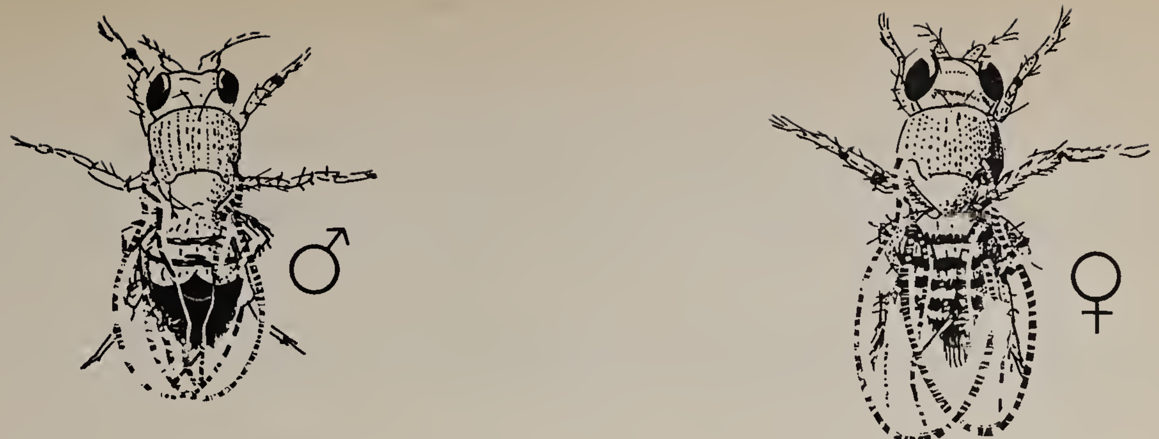
Fig. 10-2-b Transfer technique

Etherized flies will recover in about five minutes. Your study and sorting must be done quickly. If they awaken before you have completed your examination, or before transfer is completed, you can re-etherize them in the following way. Tape a strip of filter paper to the inside of the lid of a Petri dish and put a few drops of ether on the paper. Then invert the Petri dish for a few seconds over the fly that has recovered.

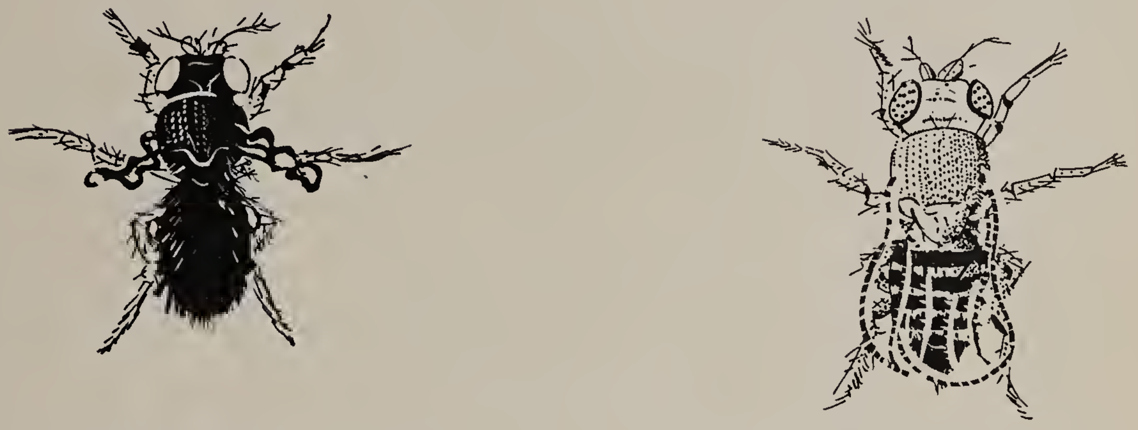
Section 4 *Identifying characteristics.* The first step in identification is to learn to distinguish the males from the females. The female has a slightly broader abdomen than the male and has small lines across the tip of the abdomen, as shown in Fig. 10-2-c. The male, which is smaller, has a black-tipped, blunt posterior end (Fig. 10-2-c). The major characteristics to learn to identify are the size and shape of the wings, the color and shading of the body, and the color of the eyes. The normal (long) wing in wild fruit flies is dominant over other characteristics such as vestigial wings, curved wings, miniature wings, and bent wings. Red eyes in the wild fruit fly are dominant over brown (sepia), black, and white eyes. The gray color of the body of the wild fruit fly is dominant over black body or yellow body. Some of the traits in the fruit fly are illustrated in Fig. 10-2-c.

Use a camel's hair brush to move the flies on the card as you study each characteristic. Avoid working under a bright light, as overheating will kill the flies. After you have sorted the flies, put each group in a fresh culture jar for further study or experimentation.

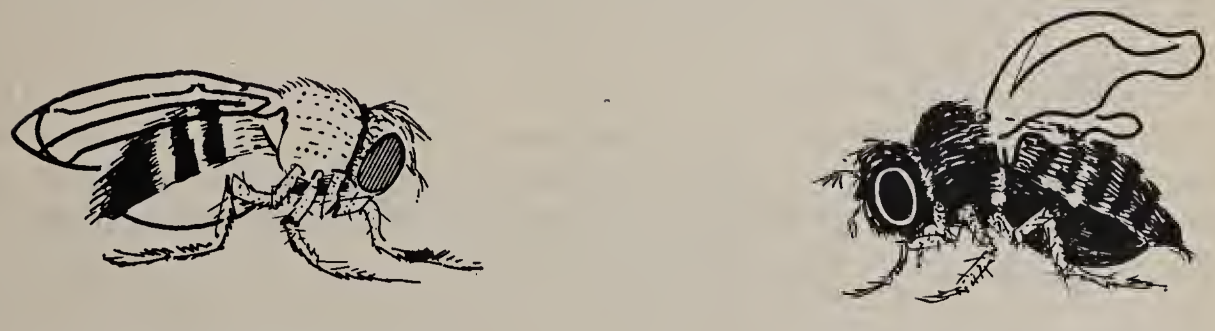
Section 5 *Crossing fruit flies.* Experimental matings must be made with females that have unfertilized eggs. These are referred to as virgin females. Select a culture jar in which a



Normal *Normal*



Vestigial wings, white eyes, black body *Miniature wings, red eyes, gray body*



Bent wings, sepia eyes, yellow body *Curved wings, black eyes, black body*

Fig. 10-2-c Characteristics in wild fruit flies

number of new adult flies are emerging. Remove *all* the adult flies and discard them or transfer them to another jar. Be sure to note when this is done. After 10 or 12 hours have elapsed, examine the jar again to see if new adults have hatched. These flies have not had time to mate and can be used in crossing experiments. Females older than 12 hours should not be used as it must be considered that they have already mated, and once mated, a female fly may not be successfully mated with another male.

Transfer these unmated flies into an etherizing jar and etherize them for examination. Separate the males and females and place two females and two males in a new culture jar. Record the date and the type of flies used (i.e., wild-type cross) on the jar. The flies will mate soon after recovering from the ether, and the females should begin producing eggs in 36 hours. In order to make sure that the experiment will be successful, it is wise to prepare three culture jars for each cross. After a few days, when a number of larvae are seen in the jars, remove the parent flies and discard them. Add a few drops of yeast suspension to supply the larvae with food and to keep the medium moist.

About 12 days after the original mating, the F_1 generation of flies should begin to emerge. The females usually hatch out first and the males appear soon after. Now put six males and six females of the F_1 generation in each of three fresh culture jars. Record the date and the type of the *original* cross. Each of these jars should ultimately contain about 250 flies for the F_2 generation. The adult flies should be removed from the three jars in about a week. As each batch of flies emerges, transfer them to an etherizing jar, then put them on a card and examine with a hand lens for the variations in wing structure, body shading, eye color, and sex. Record the number of flies having each characteristic on the table provided. Discard the flies of the F_2 after you have examined them. *Do not return them to the original jar,* as this will throw the count off. Continue your examination of the cultures daily for a week. Compute the ratio of each variation.

Date	*Sex*		*Body Color*			*Wings*					*Eye Color*			
	M	F	Gray	Black	Yellow	Normal	Vestigial	Curved	Miniature	Bent	Red	White	Black	Brown

(a) Did you have any fruit flies that had traits other than those listed on the table?

(b) If so, what were they?

Interpretation. On the basis of your data, discuss the type of body color, wings, and eye color that are dominant in the wild-type fly as indicated by the frequency of appearance in random matings.

Which of the types of fruit flies you crossed had these dominant traits?

Sex Chromosomes and Linkage 10-3

Purpose To study the inheritance of sex and of sex-linked traits.

Each human body cell contains 23 pairs of chromosomes. One of the pairs is different for the male and female and is referred to as the sex chromosomes. When a gene is carried on the sex chromosome, it is said to be sex-linked.

A Part 1 SEX CHROMOSOMES. The sex chromosomes of the female are similar and are referred to as X chromosomes. In the male the pair of sex chromosomes consists of an X chromosome and a Y chromosome.

Materials. No materials or apparatus required.

Procedure and observations. Using a Punnett square, diagram sex determination in man in the space provided. (a) What is the genotype of the female? ....

(b) the male? .... (c) What is the probability that the offspring will be male? ....

(d) be female? .... (e) Why is it impossible to predict the sex of the offspring? ....

....

Sex determination

B Part 2 STUDY OF SEX LINKAGE. In human beings, the genes controlling color vision are located on the X chromosomes. The gene for normal color vision is dominant to the gene for color-blindness. We can represent this as X^C for normal dominant color vision, and X^c for the color-blind recessive. Therefore, X^CX^c would represent a female with normal color vision who is a carrier of the recessive gene for color-blindness. A female with normal color vision who is not a carrier of the gene for color-blindness is represented by X^CX^C. X^cX^c represents a color-blind female.

In males, X^CY^- indicates normal color vision and X^cY^- indicates color-blindness. The Y chromosome does not carry a matching gene for color vision.

Materials. Four 6-inch test tubes; white pipe cleaners; 10% hydrochloric acid solution; weak brom thymol blue solution; 1% sodium hydroxide solution.

Procedure and observations. Since you cannot do experiments in human genetics, you can use a graphic demonstration to illustrate the masking of one gene by another. You will use pipe cleaners to represent the sex chromosomes and test tubes for the cells of the body. Soak pipe cleaners that will represent the X^C chromosomes in a 10% hydrochloric acid solution for about an hour. Soak the pipe cleaners that are to represent the X^c chromosomes in a 1% sodium hydroxide solution for about an hour. Leave the pipe cleaners in their respective solutions until you are ready to use them. Bend some pipe cleaners to resemble a cane. These are left untreated and represent the Y chromosome.

Fill four test tubes two-thirds full of the brom thymol blue solution. This solution serves as an indicator to demonstrate the masking of the genes when a recessive and a dominant one are present in the same cell. The solution turns yellow in the presence of an acid and blue in the presence of a base (sodium hydroxide). Number the test tube "cells" 1-4.

Distribute the variously treated pipe cleaner "chromosomes" as follows: two acid in tube 1; an acid and a sodium hydroxide in tube 2; an acid and an untreated one in tube 3; and a sodium hydroxide and untreated one in tube 4, as shown in Fig. 10-3-a.

Observe any color change that occurs in the tubes. Then record the results and conclusions in the table provided.

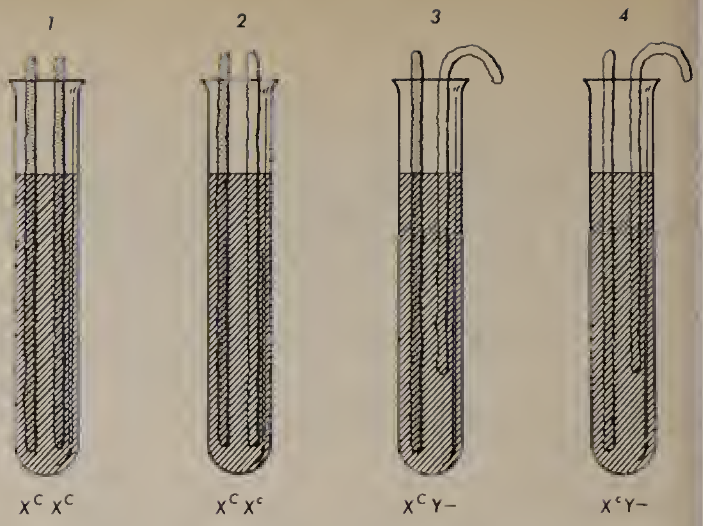


Fig. 10-3-a Technique for setting up the test tube "cells" and pipe cleaner "chromosomes"

Genes	*Color Change*	*Color Vision*	*Sex*
X^CX^C			
X^CX^c			
X^CY^-			
X^cY^-			

(a) What percent of the males in your demonstration would be color-blind? ....

(b) What percent of the females would be color-blind? .... (c) Explain your answer.

....

Work out a cross between a color-blind male and a female who is a carrier of the color-blind gene. Use a second set of pipe cleaner "chromosomes" and test-tube "cells." Prepare your own table and record your results. (d) What percent of the females would be color-blind in this cross? .... (e) Explain. ....

(f) What percent of the males would be color blind? .... (g) Explain. ....

....

Interpretation. Explain why only a small percent of the women in the population are color-blind. ....

....

....

....

C Part 3 STUDY OF SEX-LINKAGE IN FRUIT FLIES

Problem. How is eye color, a sex-linked trait, inherited in fruit flies?

You will remember from your reading that the concept of sex-linkage was first discovered in the fruit fly. The genes for red eye color and white eye color are located on the X chromosomes. The gene for red eye color is dominant over the gene for white eye color. Since the life cycle of the fruit fly is very short, you can study the F_1 and F_2 generations within a few weeks. Your observations of these generations will permit you to gain an understanding of the same principle of inheritance that Morgan first found in the fruit fly.

Suggested materials. Culture of fruit flies (red-eyed males and females); culture of fruit flies (white-eyed males and females); dissecting microscope or hand lens; small camel's hair brush; grease-marking pencil; fresh culture jars; materials for etherizing flies.

Suggested procedure. Put two or more white-eyed males and two or more red-eyed virgin females in one culture bottle and two red-eyed males and two white-eyed virgin females in a second culture bottle. Identify each culture bottle with the type of cross and date. After 7-8 days, examine the culture jars for the presence of larvae and pupae. Remove and dispose of the parent flies.

Examine both cultures daily for adult flies of the F_1 generation. Determine the eye color and sex for each of these flies. Do not return them to the cultures. Classify at least 100 flies from each cross. Terminate your study on the tenth day after the first flies begin to emerge.

Put two females and two males of the F_1 generation from each cross into separate fresh culture bottles. After 7-8 days, examine the cultures and remove the parent flies. Identify the eye color and sex of the F_2 offspring as they emerge. Examine at least 150 F_2-generation flies.

Analysis and conclusions. Summarize your results in a table. You should also diagram the crosses, showing the phenotypes and genotypes in each generation, and the eggs and sperm involved. This study should help you explain the differences you found among the F_1-generation offspring and the similarities in the F_2 offspring of the two crosses. It should also give you a better understanding of the mode of inheritance of sex-linked traits.

Purpose To determine gross, microscopic, and chemical differences in smooth and wrinkled peas.

One of the seven pairs of contrasting traits observed by Gregor Mendel in his study of garden peas was smooth and wrinkled seed coats. The fact that these characteristics appear in genetic ratios is evidence that they are hereditary. Therefore, one or more pairs of genes must be involved in producing the characteristics.

In this Investigation you will determine gross, microscopic, and chemical differences in smooth and wrinkled peas. Since these differences are hereditary in the garden pea, you will also be dealing with structural and chemical results of gene action.

A Part 1 GROSS DIFFERENCES IN SMOOTH AND WRINKLED PEAS. Why should some peas remain smooth at maturity while others become wrinkled? Is there a difference in the structure of the seed coat? Is the wrinkled condition the result of water loss? Are there internal microscopic differences in the two kinds of peas? In this Part you will examine these characteristics of smooth and wrinkled peas.

Materials. Thirty smooth peas; 30 wrinkled peas; two 250-ml beakers (or wide-mouth bottles); microscope; slides; cover glasses; platform balance; razor blade; glass-marking pencil; paper towels.

Procedure and observations. Section 1 *Gross differences associated with smooth and wrinkled peas.* Count out and weigh 30 smooth and 30 wrinkled pea seeds. Record their initial weight in the table. Identify two beakers as *smooth* and *wrinkled* with a glass-marking pencil. Put the peas in their respective beakers. Fill the beakers almost full of water and set aside until the next day.

On the following day, identify two paper towels as *wrinkled* and *smooth.* Pour off the excess water from both beakers and empty the seeds onto their respective towels. Blot the surfaces of the seeds dry with paper towels. Then weigh them. Record the weights of both sets in the table. Determine the weight increase in both sets of seeds and calculate the percentage of increases in weight.

Kind of peas	*Initial weight of dry peas*	*Weight after soaking 24 hrs*	*Percent of weight increase*
Smooth			
Wrinkled			

(a) Describe any change in the physical appearance of the two kinds of peas after soaking.

....

(b) Which group of peas showed the greatest percentage of weight increase? ....

....

(c) Account for this increase in weight. ....

....

(d) From your observations, which kind of peas would lose the most water as they mature and dry? ....

....

(e) Do the results of your observations of gross differences in smooth and wrinkled peas indicate differences in texture of the seed coat, or internal differences? ....

Now you will determine whether there are microscopic differences between the two types of peas.

Section 2 *Microscopic examination of smooth and wrinkled peas.* Mark one side of a microscope slide with an S for smooth and the other with a *W* for wrinkled. Put a drop of water on either end. Cut through a soaked smooth pea with a razor blade. Scrape the cut surface of the cotyledon and place the scrapings in the drop of water on the side of the slide marked S; mix well. Clean the razor blade and repeat the process with a soaked wrinkled seed, using the end of the slide marked *W*. Examine both ends of the slide under low power and high power.

(f) Describe the starch removed from the smooth pea. ....

.... (g) from the wrinkled pea. ....

....

Draw several starch grains from the smooth pea and from the wrinkled pea in the space provided.

Starch grains (H.P.)
smooth pea

Starch grains (H.P.)
wrinkled pea

Prepare additional slides of starch grains from smooth and wrinkled peas and determine whether the difference in the starches are characteristic of these varieties. (h) Are the differences in starch grains characteristic of all of the peas you examined? ....

(i) What conclusion might you draw from this observation? ....

....

B Part 2 **CHEMICAL DIFFERENCES IN PEAS.** As you summarize the observations you made in Part 1, evidence seems to point to further differences in smooth and wrinkled peas. Water-absorbing properties are different. The form of starch grains in the two peas is different. These variations suggest chemical differences. Chemical processes are associated with enzymes. Is there, therefore, a difference in one or more enzymes produced in the two varieties of peas?

The procedure outlined in this Part is precise and somewhat involved. However, the results should be rewarding as we continue our search at the chemical level in smooth and wrinkled peas.

Materials. Twelve gm dry smooth peas; 12 gm dry wrinkled peas; mortar and pestle; dropping bottle or beaker; Petri dishes; balance; 2-in. funnel; incubator; refrigerator; cheesecloth; granulated agar; 1 gm glucose-1-phosphate to 100 ml of distilled water.

Procedure and observations. Boil the mixture with constant stirring until the foam looks coarse. Pour sufficient glucose agar into each sterile Petri dish (previously sterilized in an oven at 350°F for two hours) to form a layer 5 mm deep. Allow to gel. Cover and refrigerate until needed. Remove the plates from the refrigerator about an hour before use.

Weigh out 12 gm of dry smooth peas and 12 gm of dry wrinkled peas. Grind the smooth peas in a mortar and pestle until a fine powder is formed. Add 12 ml of distilled water and mix thoroughly. Filter this mixture through several layers of cheesecloth into a dropping bottle or beaker. Label the bottle or beaker *smooth pea seeds.* Discard the cheesecloth. The extract you have is your "starch-producing enzyme."

Repeat the process using a clean mortar and pestle, cheesecloth, and wrinkled peas. Label the extract which you prepared from the wrinkled peas.

Divide your Petri dishes into fourths by drawing lines with the glass-marking pencil on the outside of the bottom dish. Put a drop of each of the two extracts on each section of the gelled agar plates. Use a separate plate for each extract. Be sure to identify each plate as smooth or wrinkled. Allow 30 minutes for the extracts to act on the glucose in the agar. (If an incubator is available, incubate the plates for 30 minutes at 37°C. This will speed up the action.)

After 30 minutes, add a drop of iodine to two drops of smooth-pea seed extract and to two drops of wrinkled-pea seed extract. Observe the reaction which takes place. (a) What happens to the drops of smooth-pea seed extract? ....

(b) of wrinkled-pea seed extract? .... Since there may be some starch grains present in the extract, these should be removed. Wipe off the extract and iodine with filter paper to remove the starch grains. Now examine the agar for a blue color which should remain below the surface. (c) Do you find a blue color? ....

(d) If the blue color is present, what substance has been formed? ....

After 60 minutes test the remaining two drops of each kind of pea seed extract. (e) What differences, if any, did you find in the amount of starch formed by smooth peas and wrinkled peas? ....

....

.... (f) What does this tell you about the starch-producing enzyme in the two kinds of seeds? ....

....

Interpretation. Based on the action of the extract from smooth and wrinkled peas on glucose agar, what conclusion can you draw concerning the chemical difference in the two kinds of peas? ....

C Part 3 INFLUENCE OF AN ENVIRONMENTAL FACTOR ON GENE ACTION

Problem. How does the environment affect the expression of a gene?

The organism inherits genes which determine its characteristics. However, the environment often determines what happens to the characteristic. For example, occasionally a green plant lacks the dominant gene for the production of chlorophyll. We refer to such plants as albinos. You can determine the effect of light on the production of chlorophyll by allowing the seeds produced by hybrid corn plants carrying a dominant gene for chlorophyll production and a recessive one for albinism to germinate in two different environments.

Suggested materials. Grains of corn produced from a monohybrid cross in which both parents are known to be heterozygous for albinism; Petri dishes; shoe box or light-free covering; paper toweling or filter paper.

Suggested procedure. Plant the hybrid corn seeds on damp paper toweling or filter paper placed in the bottom of Petri dishes. Cover the Petri dishes and place them in a well-lighted area of the room. Cover half of the dishes with a shoe box or other lightproof covering. The covered seeds are the experimentals, and the uncovered ones are the controls.

Check your seeds daily for evidence of germination. When the seeds begin to germinate, keep a daily record of the number and kinds of seedlings for both groups. After about half of the experimental seeds have germinated, remove the shoe box or lightproof covering. Allow the seeds to continue germinating in the light. Continue to count and record the appearance of the seeds as they germinate.

Analysis and conclusions. Your data of the number of kinds of plants germinating on a given day should be summarized in a table. The data on the uncovered group should enable you to form some conclusions relating to the hereditary control of chlorophyll production in plants. The data on the covered group and the changes that occurred after they were uncovered should help you to understand how the expression of a gene-controlled trait may depend on the proper environmental condition. You may also use the data you obtained to show the ratio of inheritance of the two traits.

Frequency of Human Genetic Traits

Purpose To study the inheritance of certain human traits and to determine their frequency in a population.

In studying human genetics it is not possible to determine the results of experimental crosses, as it was for Mendel in his study of garden peas. It is possible, however, to sample a human population to determine the frequency of a given trait and the way it is inherited.

A Part 1 INHERITANCE OF THE ABILITY TO TASTE PHENYLTHIOCARBAMIDE. The ability or inability of an individual to taste PTC as a bitter sensation is determined by genes. About 70 percent of the population are PTC tasters, and about 30 percent are nontasters.

Materials. PTC (phenylthiocarbamide) papers.

Procedure and observations. PTC papers are used in this Part to determine whether members of the class or their families are tasters or nontasters. Put one of the PTC papers in your mouth and determine whether you are able to taste it. Record your results in the table provided.

Reaction	*You*	*Your family*	*Class*	*Families of class*	*Total*	*Ratio*	*Ratio expressed as decimal*
Taster							
Nontaster							
Total							

Take some of the PTC papers home and test members of your family. Be sure to record the members as tasters and nontasters. Complete the table provided, recording the results of the class test, the members of their families, the ratio of tasters to nontasters, and the ratio expressed as a decimal. (a) On the basis of your results, can you determine whether the ability to taste PTC is due to a dominant or a recessive gene? ....

(b) If so, is the ability to taste PTC dominant or recessive? ....

(c) Could two parents who are nontasters have a child who is a taster? .... (d) Explain your answer. ....

.... (e) Could a parent who is a taster and one who is a nontaster have children who are tasters? .... (f) Explain your answer. ....

....

.... (g) Could the parents in *e* have children who are nontasters? .... (h) Explain your answer. ....

....

Prepare a pedigree of your family's ability to taste in the space provided.

A Part 2 **INHERITANCE OF THE ABILITY TO ROLL THE TONGUE.** The ability to roll the tongue is inherited as a dominant trait. People can either roll the tongue easily or not at all.

Materials. No materials or apparatus required.

Procedure and observations. First determine whether you can roll your tongue and record the result in the table. Now determine the ability of the members of your class to roll their tongues and record the result in the table. Check the members of your family to determine whether they have the inherited ability to roll their tongues. Prepare a pedigree of the inheritance of the trait in your family in the space provided.

Count the occurrence of the trait among ten individuals in your community. *Be sure that no other member of the class has counted them in their sampling.* Record your results in the table.

(a) How close is your ratio to the 3:1 ratio Mendel found in making monohybrid crosses with simple dominance? .... (b) If the ratio is not close to 3:1, give a possible explanation. ....

.... (c) Are all the tongue rollers in your survey homozygous for the trait? .... (d) Explain your answer. ....

....

Trait	You	Class	Neighborhood	Total	Ratio	Ratio as decimal
Tongue roller						
Nonroller						
Total						

Pedigree for ability to taste

Pedigree for ability to roll the tongue

A Part 3 INHERITANCE OF BLOOD TYPES. Geneticists probably know more about blood types than any other inherited human trait. The four basic blood types are determined by the presence or absence of the A and B agglutinogens in the red corpuscles. For clarity, consider blood types as being determined by a single pair of genes. Thus, type-A blood may be homozygous I^AI^A or heterozygous I^Ai. Type-B blood may be homozygous I^BI^B or heterozygous I^Bi. Type-O blood must be homozygous ii, and type-AB is a combination of A and B antigens, with the gentoype I^AI^B, illustrating co-dominance.

Materials. No materials or apparatus required.

Procedure and observations. Using the table provided, determine all possible genotypes of the parents, all possible genotypes of the children, all possible blood types, and blood types *not* possible in children.

Blood type of parents	*All possible genotypes of parents*	*All possible genotypes of children*	*All possible blood types of children*	*Blood types not possible for children*
A and O				
B and O				
A and B				
AB and A				
AB and B				
AB and O				
O and O				

(a) On the basis of your data in the table, could you prove that a child belongs to a certain set of parents? .... (b) Explain. ....

....

(c) Could you prove that a certain child did not belong to a particular set of parents? ....

.... (d) Explain. ....

....

You might investigate the frequency of several other interesting traits in man: the cephalic index, which is determined by measuring the width and length of the head, finding their ratio, and multiplying by 100; color-blindness; free or attached ear lobes; double-jointed thumbs; hair form (curly or straight); and hair color. (e) Why is it impossible to determine whether a child belongs to a certain set of parents, but possible to determine whether he does not? ....

....

....

Variations in Organisms

13-1

Purpose To demonstrate that variations occur within a species.

We define a species as one kind of organism, distinct from all others. All members of a species have the same number of chromosomes and the same arrangement of genes on the chromosomes. In spite of these likenesses, however, there is considerable variation within a species. You have only to think of the variations among human beings. The variations that occur within a given species provide the basis for natural selection.

In this Investigation you will study some of the differences found among the same species of grasshopper, pea, and lima bean.

B Part 1 VARIATIONS IN PLANT AND ANIMAL STRUCTURES. The leaves of a tree are not all the same length, the petals of a sunflower or daisy may vary in number, the length of pods on a redbud tree will vary, and not all the members of a given species of insect have the same measurements.

Materials. Thirty male or female grasshoppers of the same species (living or preserved); 100 dried lima beans; 100 fresh green pea pods; metric ruler.

Procedure and observations. Using a metric ruler, make measurements of 30 grasshoppers of the same species and sex. Measure the length of the body, the hindleg, and femur (joint nearest the body) of the jumping leg to the nearest millimeter. Record the lengths and number of grasshoppers having similar measurements in the tables provided. For example, if five of the 30 grasshoppers have femurs whose lengths range between 16.5 mm and 17.4 mm, record the length as 17 mm and five for the number of grasshoppers. Then plot the results on graphs. Let the y-axis represent the number of grasshoppers and the x-axis represent the measurements. Calculate an average for each of the measurements.

Body length (mm)										
Number of grasshoppers										

Average body length ....

Hindleg length (mm)										
Number of grasshoppers										

Average length of hindleg ....

Femur length of hindleg (mm)										
Number of grasshoppers										

Average length of femur of hindleg ....

Now use the 100 fresh green pea pods and measure their length to the nearest millimeter. Then find the average length of the pod and compare the average with the length of the individual pods. Record your data in the table provided. Then plot the data on a graph.

Length of pod (mm)										
Number of pods										

Average length of pods ....

Measure the length of 100 dried lima beans and record the results in the table provided and graph the results.

Length of lima bean (mm)										
Number of lima beans										

Average length of beans ....

Compare the curve you obtained in graphing the length of the various parts of the grasshopper with those obtained in graphing the length of the fresh green pea pods and length of the dried lima beans. (a) In what way are the curves similar? ....

.... (b) different? ....

Interpretation. Give two major causes of variations among members of a group. ....

....

....

How are variations related to the process of natural selection? ....

....

....

A Study of Classification

14-1

Purpose To become familiar with the use of a scientific classification key as a means of identifying unknown organisms.

One of the biologist's essential skills is the ability to place an organism in its correct classification group and to identify it by name. In identifying an organism such as a fish, you might look for the presence or absence of scales, the position of the fins, shape and position of the mouth, or the length of the body.

A Part 1 USING A CLASSIFICATION KEY. The characteristics that are useful in identification may be arranged in the form of a key such as the one for game fish shown in Fig. 14-1-a.

Materials. No materials or apparatus required.

Procedure and observations. Study the terms defined below, all of which refer to structures of a fish. Certain of these terms are used in the classification key on page 100. Then examine closely one of the drawings of a fish shown on the next page. Read both statements listed under number 1 in the classification key. One of these statements should fit the fish you have chosen; the other should not. Refer to the number after the statement that fits your fish and go directly to this number in the key. Again select the statement that fits the fish you selected. Continue through the key until you come to a name after one statement. This should be the name of the fish you selected. Practice using the key to identify several of the fish shown.

Suppose you want to find the name of the fish shown in the drawing numbered 2 in Fig. 14-1-a. Look at the classification key. Note that each numbered item on the left side of the key presents two possibilities. Reading item 1 of the key, we see that our fish has no scales, or at least we cannot see any. So we go down the page to number 12 on the left hand side of the key. Our fish is not elongated or snakelike, so we go to number 13 of the key. The fish we are classifying has barbels growing from its lips and the top of its head, so we go to number 14 of the key. Since our fish has a caudal fin that is rounded, and a blunt head, we know that it is the *Bullhead catfish* (also known as *horn pout* in some parts of the country.)

Terms referring to the structure of fish

Barbel—a fleshy projection from the lips or head
Fins
  Adipose—a small fin on the top mid-line of the body near the tail fin
  Anal—a fin along the lower mid-line of the body near the tail fin
  Caudal—tail fin
  Dorsal—the fin or fins along the top mid-line of the body; may be either spiny, with hard rays ending in sharp spines, or soft and lacking spines
  Pectoral—the paired fins nearest the head, corresponding to front legs or arms
  Pelvic—the paired fins nearest the tail, corresponding to hind legs
Scales—transparent, overlapping outgrowths of the skin

CLASSIFICATION KEY TO CERTAIN FISH

1.	Body more or less covered with scales	2
	Scales lacking or too small to be seen	12
2.	Dorsal fin single	3
	Dorsal fins two or more, joined or separated	6
3.	Body more than four times as long as broad (top to bottom); front edge of dorsal fin far back on body; mouth large, hinge back of eye	4
	Body less than four times as long as broad; front edge of dorsal fin about midway between head and tail; mouth not large, hinge in front of eye	5
4.	Dark lines forming netted design on body; fins not spotted	*Pickerel*
	Body covered with yellow spots; fins spotted	*Northern pike*
5.	Mouth turned downward; barbels absent; dorsal fin not elongated	*White sucker*
	Mouth not turned downward; barbels present; dorsal fin elongated	*Carp*
6.	Two dorsal fins separated, the anterior spiny and the posterior soft	7
	Two dorsal fins united, forming an anterior spiny portion and a posterior soft portion	8
7.	Top of head concave, forming a hump in front of dorsal fin; dark vertical bars on body	*Yellow perch*
	Top of head not concave, body sloping to dorsal fin and not forming a hump; dark blotches on body	*Wall-eyed pike*
8.	Body more than three times as long as broad	9
	Body less than three times as long as broad	10
9.	Hinge of jaws behind the eye; notch between spiny and soft dorsal fin deep and nearly separating into two fins	*Large-mouth black bass*
	Hinge of jaws below the eye; notch between spiny and soft dorsal fin not nearly separating into two fins	*Small-mouth black bass*
10.	Mouth large, hinge below or behind eye	11
	Mouth small, hinge in front of eye	*Bluegill*
11.	Five to seven spines in dorsal fin; dark spots forming broad vertical bars on sides	*White crappie*
	Ten or more spines in dorsal fin; sides flecked with dark spots.	*Rock bass (Redeye)*
12.	Body much elongated and snakelike; dorsal, caudal, and anal fins continuous	*Eel*
	Body not elongated and snakelike; dorsal, caudal, and anal fins separate; adipose fin present	13
13.	Barbels growing from lips and top of head; head large and broad	14
	Barbels lacking; head not large and broad	16
14.	Caudal fin deeply forked; head tapering	15
	Caudal fin rounded or slightly indented but not forked; head blunt	*Bullhead catfish*
15.	Dorsal fin rounded at top; body silvery, speckled with black markings	*Channel catfish*
	Dorsal fin long and pointed at top; body bluish-gray without speckles	*Blue catfish*
16.	Caudal fin deeply forked; back not mottled and with few spots	*Atlantic salmon*
	Caudal fin square or slightly indented; back mottled or spotted	17
17.	Back and caudal fin spotted; broad horizontal band along sides	*Rainbow trout*
	Back mottled with dark lines; caudal fin not spotted; fins edged with white	*Brook trout*

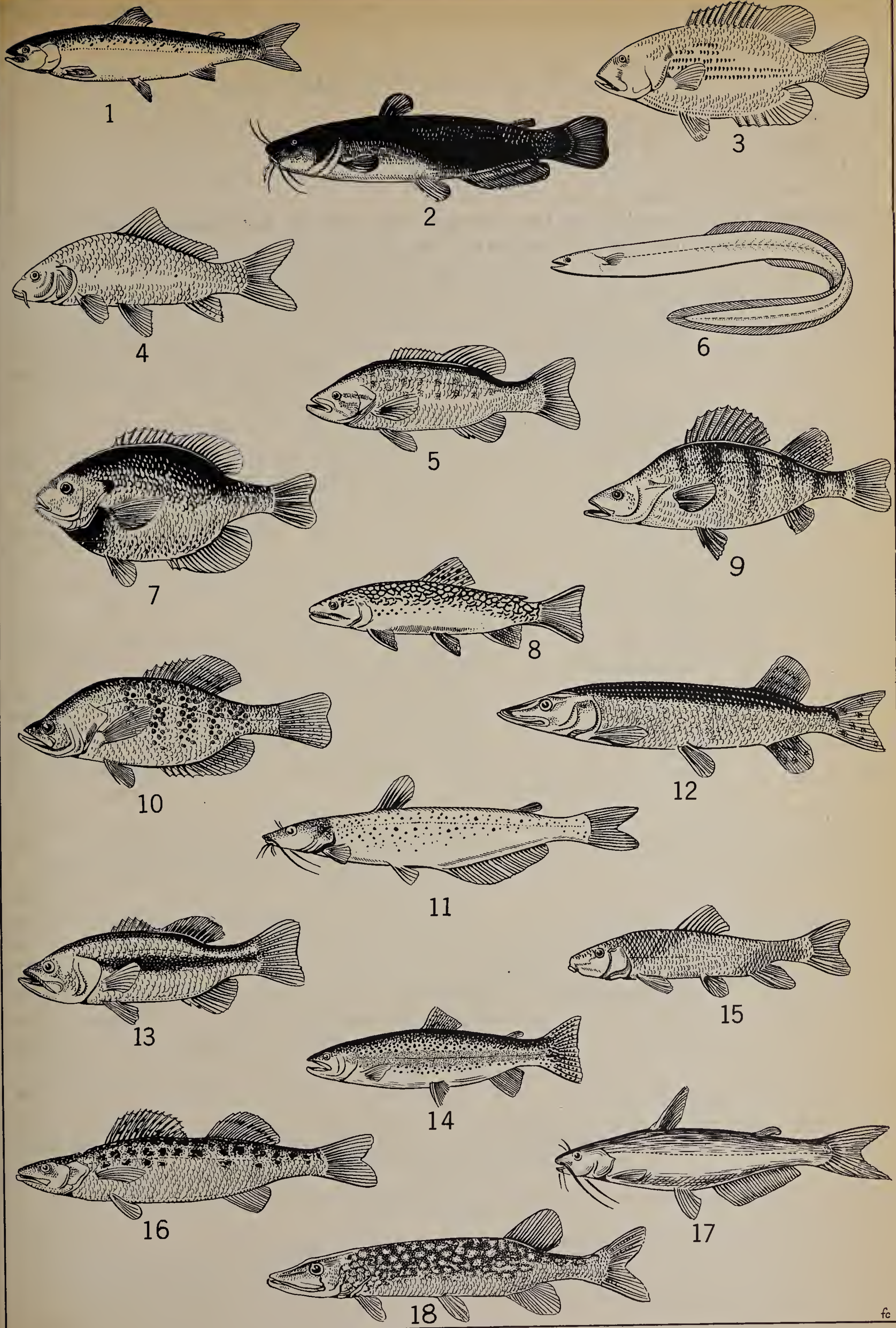

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Fig. 14-1-a Native fish

Composition and Lytic Cycle of a Bacterial Virus

15-1

Purpose To determine the structure and composition of a bacterial (phage) virus and illustrate a lytic cycle of infection of a bacterial cell.

Virus particles have certain properties of living organisms, yet lack the cellular organization characteristic of living things. They seem to shuttle back and forth between the living and nonliving state. The substances composing viruses are organic molecules synthesized by the metabolic processes of other cells. The presence of a nucleic acid, DNA, in some viruses and RNA in others tends to place them in the category of living things.

A Part 1 **STRUCTURE AND COMPOSITION OF A PHAGE VIRUS.** The electron microscope, with its magnifications of 100,000 times or more has enabled biologists to determine the structure of many viruses. Determination of the composition of viruses is important in understanding their action on host cells.

Materials. No materials or apparatus required.

Procedure and observations. Figure 15-1-a shows the structure of a phage virus. Notice that the virus resembles a tadpole with a many-sided head and a slender tail. The outer shell is composed of protein. A spiral core of DNA lies within the shell. In Fig. 15-1-a, label: **head, tail, protein shell, and DNA core.**

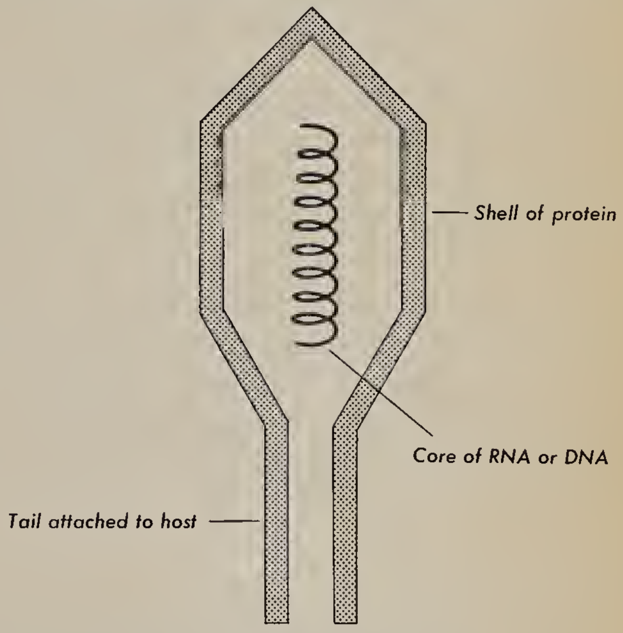


Fig. 15-1-a Phage virus

A Part 2 **LYTIC CYCLE OF A PHAGE VIRUS.** A cycle of lytic infection of a bacterial cell by a phage virus reveals certain physiological properties of a virus that tend to place it in the category of living organisms. During such a virus attack on a bacterial cell, the phage injects its DNA core into the bacterial cell through an opening dissolved in the wall. The presence of the phage DNA disorganizes the synthetic processes of the bacterium, resulting in the formation of virus particles from the substances of the bacterium. Such a cycle of lytic infection may destroy a bacterial cell in less than one minute.

Materials. No materials or apparatus required.

Procedure and observations. To illustrate a cycle of lytic infection and destruction of a bacterial cell, prepare a series of diagrams in the space provided as follows:

1. Show an uninfected bacterial cell in the form of a rod or a sausage approximately 2 in. in length.
2. Attach a phage virus, tail down, to the wall of the bacterium. The head of the phage should be approximately ¼ in. in diameter.
3. Show the DNA core of the phage injected into the bacterium and the empty shell attached to the wall of the bacterium.
4. Show numerous spirals of phage DNA within the bacterium resulting from altering of

the synthetic processes of the bacterium and production of phage DNA instead of bacterial DNA.

5. Organize numerous phage particles within the bacterial cell. The protein shells of these particles are formed from bacterial proteins.

6. Show the bursting of the bacterial cell wall and release of the phage particles.

Cycle of lytic infection of a bacterial cell by a phage virus

C Part 3 EFFECT OF *T-EVEN* PHAGES ON *ESCHERICHIA COLI*-STRAIN B

Problem. How to demonstrate the effect of *T-even* phages on *Escherichia coli*-Strain B.

Bacteriophages are extremely small particles that multiply in living bacterial cells. Since we cannot see them, what evidence can we produce that demonstrates that phages enter bacterial cells and are reproduced there? F. W. Twort and F. H. d'Herelle first observed the activity of the phages when they saw plaques (clear areas) on inoculated agar plates. They found that they spread until the entire bacterial colony had been destroyed. They also found that the agent that d'Herelle named bacteriophage could be transferred to other colonies with an inoculating needle.

The electron microscope has revealed what actually happens during the destructive attack of a bacterial cell by a phage particle. You diagrammed this lytic cycle of the phage virus in Part 1 of this Investigation. Now you can demonstrate the activity of the phage particles by inoculating a bacterial culture with the phage virus.

Suggested materials. Stock culture of *Escherichia coli,* Strain-B; stock culture of *T-even* bacteriophage; inoculating loop; seven 1-ml pipettes; 4 bacteriological pipettes graduated at 0.01 ml; one 10-ml pipette graduated in 1 ml; four 6-in. culture tubes; 8 Petri dishes; 500-ml flask; glass rod bent at 90° angle (2 inches on each side); incubator; refrigerator; nutrient broth; nutrient agar; distilled water.

Suggested procedure. Transfer a loopful of *Escherichia coli,* Strain-B to 250 ml of sterile nutrient broth. Incubate the inoculated broth for 24 hours at 35-37°C (96-98°F).

Label three culture tubes containing 9 ml sterile nutrient broth as *1, 2,* and *3.* Transfer 1 ml of *T-even* bacteriophage culture to tube 1. Diffuse the inoculum by rotating the tube between the hands. Then transfer 1 ml of this inoculum to tube 2 and diffuse the inoculum. In the same manner, transfer 1 ml from tube 2 to tube 3.

Transfer 0.01 ml of the phage culture from tube 1 to a sterile agar plate identified as *1.* Add 1 ml of *E. coli* to this same plate. Mix and spread the inoculum over the plate with a bent glass rod. Transfer 0.01 ml of phage inoculum from tubes 2 and 3 to two additional plates. Add 1 ml of *E. coli* culture to the plates; mix and spread with a bent glass rod. Introduce 1 ml of *E. coli* to a fourth plate to serve as a control. Incubate all plates for 24 hours at 35-37°C (96-98°F). Do not invert the plates.

After 24 hours examine the plates and notice the film of bacteria on the control plate. Examine the other plates and look for plaques. Each plaque contains a large number of phage particles. These are descendents of a single phage particle that infected a single *E. coli* cell and reproduced within it, causing the bacterial cell to rupture, releasing phage particles that destroyed other bacteria in the area.

To determine that the clear areas contain phage particles, use an inoculation loop to transfer a loopful of material from one plaque to a culture tube containing 9 ml of sterile nutrient broth. Put 1 ml of *E. coli* on a sterile agar plate. Then transfer 0.01 ml of the phage culture; mix and spread on the plate. Incubate for 24 hours at 35-37° C (96-98°F). After 24 hours examine the plate for plaques. These are evidence that phage particles were present in the plaques on plates 1, 2, and 3.

Analysis and conclusions. Explain how the appearance of the plaques on the agar plates is evidence that phage particles were present and multiplied within the bacterial colony to leave a visible plaque. How is this a demonstration of the lytic cycle?

The Bacteria—Forms and Motility 16-1

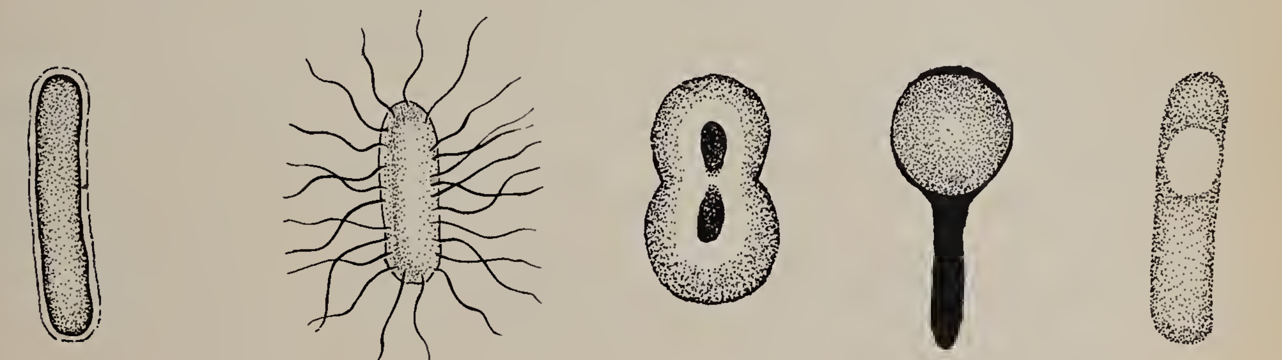
Purpose To study the morphology of a bacterial cell, forms, and grouping of bacteria and their motility.

Bacteria are very small and difficult to study under the standard light microscope. The cell lacks an organized nucleus, but the electron microscope has revealed that the nuclear materials are concentrated near the center of the cell. In this Investigation you will use diagrams to study the morphology of the cell, prepared slides or living materials for the study of forms and flagella, and living material for the study of motility.

A Part 1 STRUCTURE OF A BACTERIAL CELL. The extremely high magnifications of the electron microscope have permitted the biologist to explore the content of bacterial cells in much greater detail than was possible under the light microscope. Certain of these structures are shown in Fig. 16-1-a. The thin slime layer characteristic of bacterial cells is difficult to distinguish with the standard light microscope. However, when it is thickened sufficiently to become a capsule, it is clearly visible. Many rod-shaped bacteria produce endospores. They may be central or terminal, depending on the species.

Materials. No materials or apparatus required.

Procedure and observations. Label the following on the bacillus (nonmotile) cell: **slime layer, cell wall, plasma membrane, chromosome, cytoplasm.** Label **flagella** on the bacillus (motile) cell, **capsule** and **cytoplasm** on the diplococcus cell and **endospore** on the *Bacillus subtilis* and *Clostridium tetani* cells.



Bacillus nonmotile *Bacillus motile* *Diplococcus* *Clostridium tetani* *Bacillus subtilis*

Fig. 16-1-a Diagram of bacterial cells

(a) What is the function of the slime layer? ....

.... (b) What does the presence of a capsule indicate concerning the organism? ....

.... (c) What kind of bacteria form endospores? ....

.... (d) Explain why bacteria form spores.

....

....

A Part 2 **FORMS OF BACTERIA.** Bacteria are found in three forms; coccus, bacillus, and spirillum. These cells are often grouped together to form clusters, cubes, or chains. You can study the various forms of bacteria on prepared slides or living forms from cultures in the laboratory.

Materials. Pure cultures of living material; prepared slides of the various forms and groups of the forms of bacteria; microscope; slides; cover glasses.

Procedure and observations. Using the prepared slides or living cultures, study the various forms of bacteria. Study the cells under low power and high power of the microscope. (a) How do they appear under low power? ....

(b) Can you distinguish any bacteria under high power? .... Examine the slide carefully. (c) How do the bacteria on your slide differ from one another? ....

.... (d) Using the technique described in Investigation 1-2, determine the comparative size of the three forms. ....

....

Draw the different forms of bacteria in the space provided, identify the organism as to coccus, diplococcus, streptococcus, staphylococcus, tetrad, bacillus, streptobacillus, or spirillum, and list the form to which each organism belongs.

Organism	Organism	Organism	Organism ....
Form	Form	Form	Form ....

Organism	Organism	Organism	Organism ....
Form	Form	Form	Form ....

A Part 3 STUDY OF MOTILITY IN A HANGING-DROP PREPARATION. Bacteria that are suspended in air or liquid move with an oscillating or bounding motion referred to as *Brownian movement.* This haphazard motion is caused by collisions between the moving molecules of the medium and the bacteria. True motility is exhibited by bacteria with flagella. Flagella are cytoplasmic extensions of the cell which move a cell by a wavy or corkscrew motion. Coccus cells never have flagella, so that true motility is limited to bacillus and spirillum forms.

You can study motility in bacteria by preparing an infusion in which bacteria grow and examining the infusion in a hanging drop.

Materials. Fifteen to twenty dried beans; 400-ml beaker; microscope; culture (concave) slides; hanging-drop (25 mm) cover glasses; petroleum jelly; Bunsen burner; tripod; inoculating loop; toothpicks.

Procedure and observations. To grow bacteria for examination in the living condition, prepare a bean infusion by putting 15-20 beans in half a beaker of water and boil them for a few minutes. Add water to fill the beaker. Set the infusion in a warm place, exposed to the air for two or three days. Transfer a loopful of the infusion to the center of a hanging-drop cover glass. Using a toothpick, place a thin film of petroleum jelly around the edge of the concavity of the culture (concave) slide. Hold the culture slide, hollowed side facing down, over the drop. Press the slide gently against the cover glass so that the petroleum jelly will spread and make contact. Invert the slide and examine under high power of the microscope. The infusion should be teeming with bacteria. Darken the field by closing the diaphragm, and the organisms will be more visible. (a) Which forms are most common? .... (b) Do you find colonies? .... (c) If so, what kind are they? .... (d) Are motile (free-swimming) bacteria present in the infusion? .... (e) What type of motility is demonstrated in your culture? .... (f) Do the cells move individually or in groups? .... (g) What cell structures enable many bacteria to move in fluid surroundings? .... (h) Can you distinguish any flagella on the cells? ....

Laboratory Culture of Bacteria

16-2

Purpose To become familiar with bacteriological techniques, to prepare bacteriological culture medium and slants and Petri-dish cultures, to inoculate cultures, to incubate the cultures, and to record the appearance of the bacterial growth after incubation.

Although bacteria are found everywhere, they cannot be studied unless they are grown in large numbers in the laboratory. In order to culture bacteria, a culture medium must be provided that contains essential substances for nourishment and the physical environment necessary for their growth. Some bacteria can grow in or on many kinds of media; others are highly specific and require special media for growth.

In your study of bacteria, you will make use of several types of glassware in which they can be cultured. These include Petri dishes, culture (test) tubes, and Florence and Erlenmeyer flasks. All glassware must be thoroughly cleaned and sterilized before using. The medium must also be sterilized to free it of microorganisms. The bacteriologist usually puts the medium into culture tubes and flasks before sterilization. Then the flasks and tubes and their contents are sterilized. However, the medium is poured into Petri dishes after sterilization.

In this Investigation you will use the same techniques used by the bacteriologist when he cultures bacteria for study. It is important that you practice sterile techniques and perform each step of the Investigation with extreme care. Remember that, while you are dealing primarily with harmless bacteria, pathogenic organisms might be introduced into your cultures. (CAUTION: *Handle all cultures as though they contained dangerous organisms.*)

A Part 1 PREPARATION AND STERILIZATION OF CULTURES. For your work in bacteriology you will use commercially prepared medium, obtained from a biological supply house. The amount of culture medium you will prepare will make approximately 30 Petri-dish cultures (100 mm dishes), and fill 30 culture tubes (⅝ by 5 or ¾ by 6 in.) one-third full to be used for slants. Thus, each person in a class can inoculate at least one slant. Then, all glassware and media must be sterilized. It is absolutely necessary that *all materials used in the study of bacteria be sterilized.*

In this Part you will become familiar with the preparation of medium for culturing bacteria and make use of the sterile techniques used by the bacteriologist when he grows bacteria for study.

Materials. Dehydrated nutrient agar; 1000-ml Erlenmeyer flask; culture (test) tubes (enough for the class); Petri dishes (number to be determined by the class); wire basket; balance; clean cloth; paper towels; cotton; large spoon or spatula; pan; hot plate; funnel; rubber tube with clamp; ring stand; pressure cooker or autoclave; hot-air oven; distilled water.

Procedure and observations. Prior to the preparation of the nutrient agar (previous day), wash the Petri dishes, rinse thoroughly, and allow to dry. Shine the Petri dishes and place them, with covers in place, in a hot-air oven and sterilize them at 350°F for two hours. Leave them in the oven or stack them on a table or shelf and cover with a clean cloth or paper towels until you are ready to use them.

Commercially prepared nutrient agar (available at supply houses) is more easily obtained than the various ingredients for preparing the medium in the laboratory. Read the directions for adding water provided on the label. Weigh the proper amount for making one liter (1000 ml) of medium and put it in a pan. Add 1000 ml of water (distilled water is preferable). Stir thoroughly and put over a burner. Stir constantly, using a large spoon or spatula, until the solution comes to a boil. Be sure that all the agar has dissolved. Pour half of the medium into an Erlenmeyer flask and plug the mouth of the flask with cotton. Put the other half in a funnel equipped with a rubber tube and clamp for controlling the flow through the tube, or pour it into another flask for pouring into tubes. Fill the culture tubes one-third full of medium and plug them with cotton. The plugs should fit securely and should extend about one inch into the tube and one and one-half inches out of the tube. Avoid getting medium on the upper walls of the tube or around the mouth. Set them upright in a basket. Do not allow the medium to contact the cotton plug.

Put the plugged flask of medium and the basket of plugged tubes in a pressure cooker or autoclave and sterilize at 15 lbs for 15 minutes. In operating the pressure cooker, be sure that you leave the valve open until a stream of steam is given off. The presence of air in the chamber may interfere with sterilization.

After sterilization, allow the medium to cool slightly but not to gel. Remove the tubes from the sterilizer and lay them, individually, in a slanting position so that the agar extends from the bottom of the tube to a point about one inch below the cotton plug. The tube should not be slanted so much that medium touches the cotton plug. The entire bottom of the tube should be covered with medium. Allow the tubes to cool to room temperature. These tubes are referred to as slants, since the agar has gelled in the tube in a slanting position.

Arrange the sterilized Petri dishes in piles of about four each. Wash your hands thoroughly. Remove the flask of medium from the sterilizer and take out the cotton plug. Flame the mouth of the flask. Pour the dishes by raising the cover slightly and adding enough sterile medium to cover the bottom of each dish to a depth of about one quarter of an inch. Replace the cover as quickly as possible. Even though some bacteria may be introduced during pouring, this method is superior to sterilizing the dishes after pouring, since sterilization usually splashes medium against the cover of the dish. Put the dishes on a level surface and allow them to gel.

A Part 2 **INOCULATION, INCUBATION, AND EXAMINATION.** The technique of transferring bacteria from one source to another without contamination is basic to all your work with bacterial cultures. You will need to develop a steady hand in order to transfer the bacterial cells quickly and smoothly. In this Part you will become familiar with the various techniques used in transferring bacteria with an inoculating needle or loop to sterile, gelled nutrient agar plates and slants. You will also become familiar with incubation and examination methods.

Materials. Stock cultures of harmless bacteria (*Serratia marcescens* or *Sarcina lutea*); sterile slants; poured Petri dishes (sterile agar); inoculating needle; Bunsen burner; glass-marking pencil or gummed labels; incubator; milk; stagnant water.

Procedure and observations. Culture tube slants are inoculated by means of an inoculating needle which can be made by inserting a platinum or nichrome wire (2-3 in. long) in a heated glass rod or metal handle. If you cannot make an inoculating needle, use a dissecting needle for inoculating. Slants should be inoculated as follows; turn the cotton plug to be sure that it will slip out easily; sterilize the inoculating needle by passing it through a flame; touch the sterilized needle to the source of bacteria; holding the slant in your left hand, remove the plug by grasping it between the bent little finger and the palm of the right hand (you are holding the needle with the thumb and forefinger of the same hand); flame the mouth of the tube; insert the needle into the tube and lay the point against the lowest part of the medium; make a zigzag line along the surface of the medium from the bottom to the top; flame the needle and the mouth of the tube and replace the cotton plug. Inoculate one or more slants with each of the following, using the above procedure: milk, stagnant water, and a stock culture of harmless bacteria, such as *Serrätia marcescens* or *Sarcina lutea.* With a stock culture, use the same technique in handling the tube as outlined for inoculation. Mark the source of each inoculation with a label, fastened near the top of the tube, or with a glass-marking pencil.

The Petri dishes will be inoculated in three ways:

A. Streak plates. Sterilize the inoculating needle and put the tip of it on the material to be added to the dish. Open the dish quickly and hold the cover close to the dish in your left hand. Touch the needle to the medium in the upper left area of the dish. Draw a line from left to right. Move the needle down about one-half inch and make another line, parallel to the first. Continue to the bottom of the dish. Now turn the dish one-quarter turn to the right. Sterilize the needle and make another series of parallel lines at right angles to the first. This procedure will spread the bacteria over the plate. Inoculate two dishes from each source used in inoculation of the slants. Label the dishes.

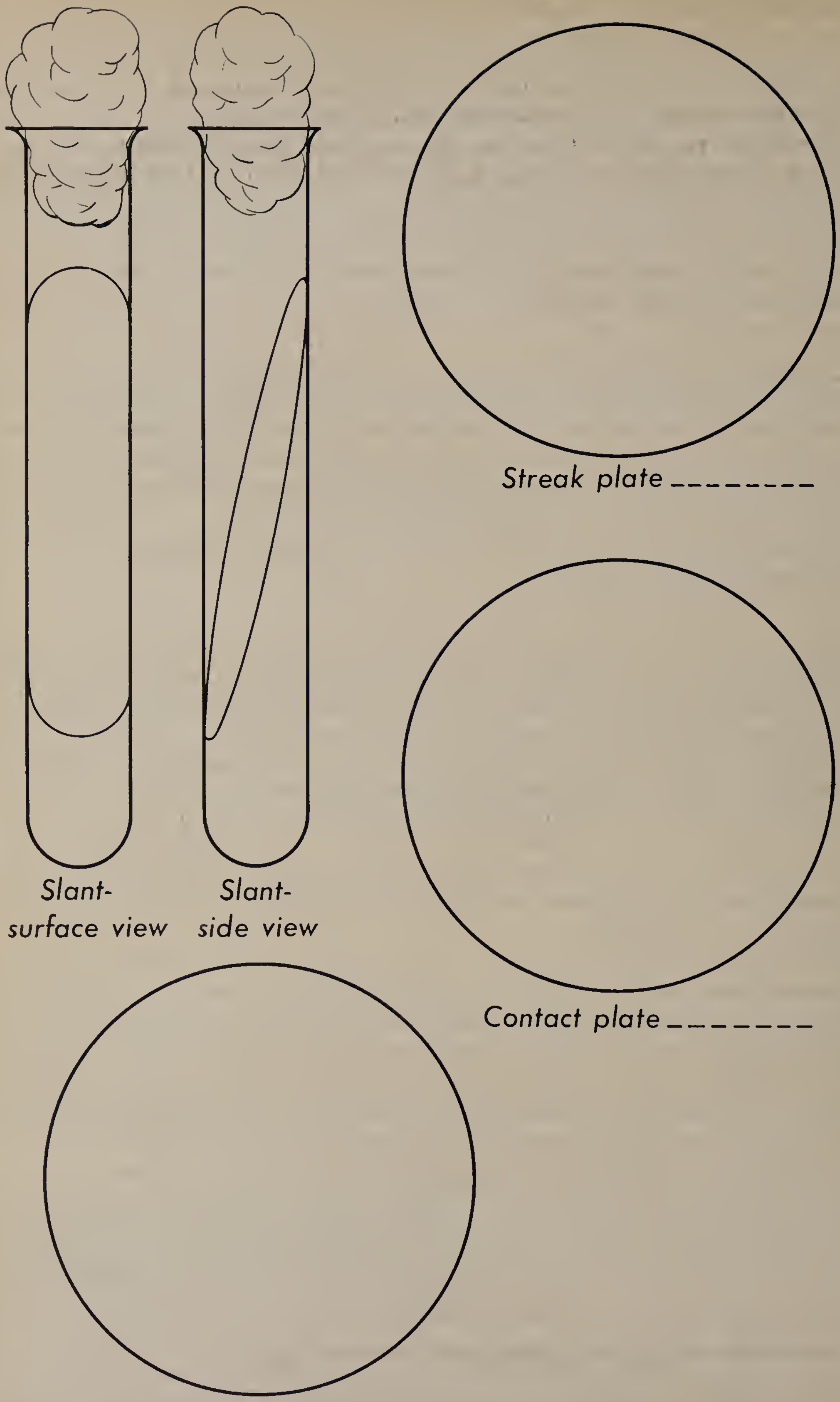
B. Contact plates. Contact plates are made by touching an object to be tested to the surface of the medium in a Petri dish. Catch a fly and put it in a Petri dish by raising the cover slightly. Allow the fly to walk over the surface of the medium, then release it. Inoculate another dish by pressing several fingers against the medium. Cover immediately. Using a third dish, press a coin against the medium and drop it out by inverting the dish. Replace the cover immediately. Make duplicates of the above inoculations. Label each.

C. Exposure plates. Open two of the dishes, laying the cover beside the dish. Expose the medium to the air for ten minutes. Inoculate two other dishes by coughing on the surface of the medium. Cover immediately.

D. Control plate. At least one dish should be left uninoculated as a control. This plate should not produce growth, except for a small number of colonies which may have been introduced in pouring. If too many bacteria develop, the cultures were not sterile prior to inoculation.

Put the inoculated slants and Petri-dish cultures in an incubator operating at 35-37°C or, if an incubator is not available, in a dark, warm place. Incubate for 48 hours. At the end of this time, individual bacteria, added during inoculation, should form visible colonies.

Examine the growth of bacteria on the slant. Notice the color of the mass, the nature of the growth on the surface of the medium, and the depth to which the bacteria have penetrated the agar. Show a surface view and a side view of your slant in the tubes outlined in Fig. 16-2-a. Examine the Petri-dish cultures. The streak plate should show lines of growth where the needle contacted the agar. Colonies of bacteria should be concentrated in the area where the object tested touched the surface of the medium in the contact plate. The colonies that developed in the exposure plate should be distributed over the surface of the medium. Notice variations in the color, size, shape, elevation, and margins of the different colonies. Show the growth of bacteria on the three kinds of plates in the outlines provided in Fig. 16-2-a. Under each plate indicate the source of the bacteria introduced.



Slant-
surface view

Slant-
side view

Streak plate ________

Contact plate _______

Exposure ________

Fig. 16-2-a Bacteria cultures

Purpose To collect bacteria from common objects and the atmosphere and to determine the effect of weather conditions on the bacterial count of the atmosphere.

Although we cannot see them, bacteria are found almost anywhere and are the most abundant organisms. Any environment that can support life in any form has its own population of bacteria.

A Part 1 COLLECTING BACTERIA FROM VARIOUS OBJECTS. One way to demonstrate that bacteria are universal in habitat is to collect them from various everyday objects and culture them. This is done by making *contact plates.* The objects are touched to sterile agar plates and incubated so that the bacteria can multiply and produce a visible colony.

Materials. Petri dishes (sufficient in number to make several contact plates); glass-marking pencil or gummed labels; balance; funnel; rubber tube with clamp; ring stand; pressure cooker or autoclave; hot-air oven; incubator; pan; hot plate; colony counter; nutrient agar; distilled water.

Procedure and observations. The directions for preparing culture medium and sterilization techniques are given in Investigation 16-2. Pour approximately 20 ml of sterile nutrient agar into each of the sterile Petri dishes and allow to gel.

Now you are ready to inoculate the plates. Use an uninoculated plate as a control. The following are suggested as possible subjects for inoculations: lipstick, powder puff, pencil eraser, doorknob, spout of drinking fountain, drinking fountain handle, coin, folded dollar bill, fingers, necklace, earring, key, chewed gum, or lip prints.

Slowly lift the lid of the Petri dish and touch your fingertips to the medium or place an object briefly on it. The lid is raised slowly so as to reduce the air currents, which may carry dust into the dish. Hold the cover directly above the dish to keep dust from falling out of the air into the dish. Close the dish immediately after inoculation, label and incubate with the control plate for 48 hours at 35-37°C. During incubation, the bacteria that came off the object will reproduce and form visible colonies.

After 48 hours, check your results. (a) Do you find colonies in every plate? ....

(b) If not, explain. ....

....

Study the colonies under the low power of the microscope. These can be observed through the bottom of the inverted plate. (c) Do you find any organisms in addition to the colonies of bacteria? .... (d) If so, what are they? ....

(e) Account for their presence in the Petri dish. ....

....

(f) Which of the subjects you chose produced the greatest number of colonies? ....

.... (g) the fewest? .... (h) Explain the variation in the number of colonies on the individual plates. ....

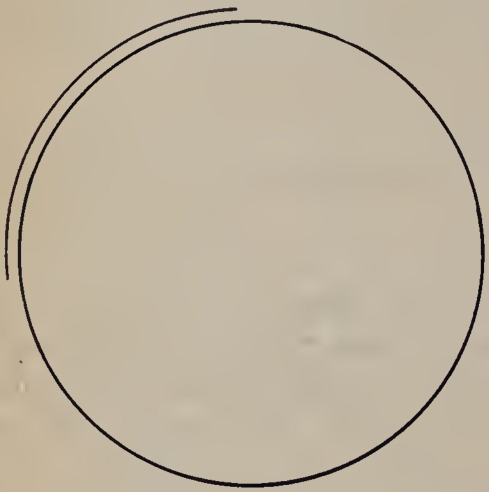
....

Make a drawing of two of the contact plate cultures in the outlines provided. Indicate under each one the source of the bacteria.

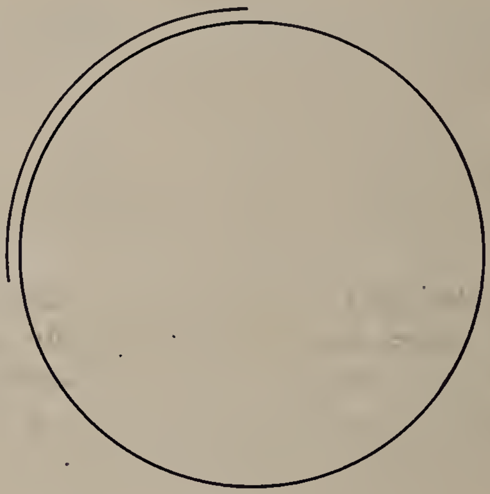
A Part 2 **BACTERIA IN THE AIR.** A second method of demonstrating the universality of bacteria is to make an *exposure plate.* In this Part you will check the bacterial content of the air in various places. The places of exposure may include your own laboratory, a crowded corridor, cafeteria, auditorium during assembly, gymnasium, locker room, and study hall. Always include an exposure made outdoors. Be sure to use a control plate for each area.

Materials. Petri dishes (sufficient number for several exposures); glass-marking pencil or gummed labels; balance; funnel; rubber tube with clamp; ring stand; pressure cooker or autoclave; hot-air oven; incubator; pan; hot plate; colony counter; nutrient agar; distilled water.

Procedure and observations. To make an exposure, open each Petri dish for 15 minutes. Put the cover beside it. Mark each dish after exposure, indicating the place of inoculation. Incubate for 48 hours at 35-37°C. During incubation the bacteria that fell on the agar will multiply and form visible colonies. Since the area of a standard Petri dish (100 mm by 15 mm) is 1/15 of a square foot, the number of colonies you count represents the number of bacteria falling per square foot per minute in a 15-minute exposure. If your Petri dish is not standard, or if you use a different exposure time, it will be necessary for you to calculate the portion of a square foot your dish represents and adjust the exposure time factor to determine the number of bacteria falling per square foot per minute.

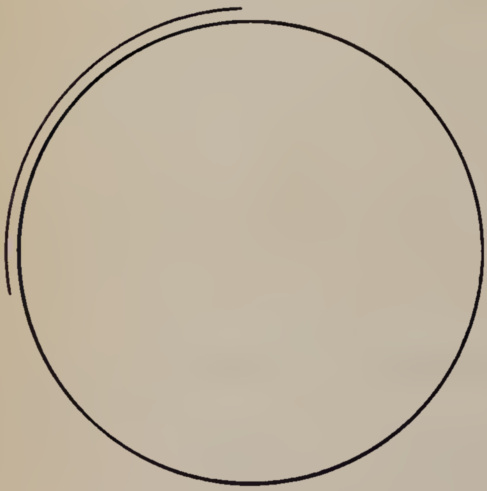


Object..

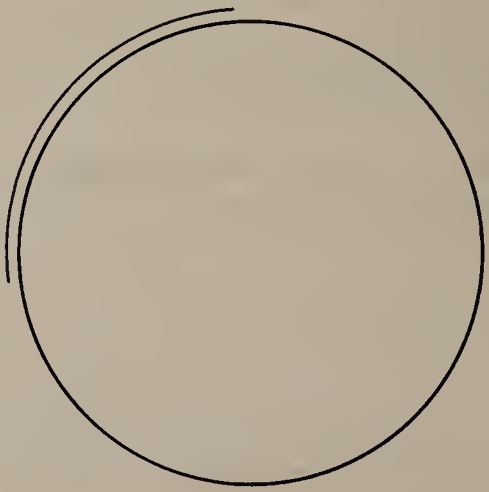


Object..

Contact plates



Area ....
Bacteria falling per sq. ft. per minute



Area ....
Bacteria falling per sq. ft. per minute

Exposure plates

Count the colonies on each of the plates and record your data. Draw two of the exposure plates in the outline provided. Indicate the area in which each was exposed and the number of bacteria falling per square foot per minute.

(a) Which exposure had the most colonies? .... (b) the least? ....
(c) How did the count in the culture exposed outdoors compare with the other exposures?

.... (d) Explain the variation in number of bacteria falling per square foot per minute in the areas in which you made exposures. ....

.... (e) List the conditions that might affect the number of bacteria falling per minute per square foot outdoors. ....

....

C Part 3 EFFECT OF WEATHER CONDITIONS ON THE BACTERIAL COUNT OF THE ATMOSPHERE

Problem. How do variations in temperature, relative humidity, air pressure, wind velocity, precipitation, and light rays affect the bacterial population of the atmosphere?

We often associate the prevalence of airborne infections with weather conditions at different seasons. Would this suggest that variations in atmospheric conditions change the bacterial population of the atmosphere? This may be determined by using the exposure technique you have learned and correlating the results with meteorological data.

Suggested materials. Sterile Petri dishes poured with sterile nutrient agar (3 per day); incubator; colony counter; glass-marking pencil.

Suggested procedure. Establish three outdoor stations in your home or school community where plates may be exposed daily for a period of two to four weeks. Expose the plates in at least three stations for a period of 15 to 30 minutes. All daily exposures should be made at the same stations for the same length of time and at the same time of day.

It will be necessary to determine the air temperature, air pressure, wind velocity, relative humidity, precipitation, and amount of light (sunny, partly cloudy, cloudy, overcast). You may make these determinations if weather instruments are available; otherwise, consult your local weather bureau.

Make each count after 48 hours of incubation and determine the average count of the three daily exposures. From this average plate count, determine the bacteria falling per square foot per minute.

Analysis and conclusions. Summarize your bacteriological and weather data in a table and a series of graphs. From your data, you should be able to draw valid conclusions concerning the correlation between bacterial count in the atmosphere and weather conditions.

Staining Bacteria 16-4

Purpose To become familiar with various stains and staining reactions used in bacteriology and the techniques for staining bacteria for microscopic examination.

Staining is an important phase of the study of bacteria in the laboratory. Living bacteria are nearly colorless and would be difficult to see clearly under the microscope without a stain to emphasize cell shapes and structures. *Simple stains,* which color the entire cell, are used to make these details clearly visible. Further use is made of stains in classifying and identifying bacteria on the basis of their staining reactions. These stains are known as *differential stains.* Gram's stain is a universally used differential stain. In classifying and identifying many bacteria, a bacteriologist may need to examine spores and their location in cells or determine the number and location of flagella. These determinations require still other *special stains.*

A Part 1 STAINING BACTERIA. In the Sections of this Part, you will become familiar with the uses of several types of stains and the techniques for preparing stained smears for microscopic examination.

Materials. Bean infusion or a nutrient-broth culture of a flagellated bacterium; bacteria cultures (agar slants or Petri-dish cultures prepared in Investigation 16-2 or 16-3); microscope; slides; cover glasses; Bunsen burner; paper towel; cover-glass holder; inoculating loop; simple stain (crystal violet, methylene blue, or safranin); Gram's differential stains and reagents (crystal violet, 5% sodium bicarbonate solution, Gram's iodine solution, 95% alcohol or acetone, carbol fuchsin or safranin); spore stains and reagents (carbol fuchsin, 5% acetic acid solution, methylene blue); distilled water.

Procedure and observations. Section 1 *Use of simple stains.* Mount a clean cover glass in a holder. With a flamed inoculating loop, place one loopful of distilled water in the center of the cover glass. Flame the loop again and transfer a small number of bacteria from an agar slant or a colony on a Petri dish culture to the drop of water. Spread the bacteria in a thin film on the cover glass. If you use a bean infusion, place one loopful of the infusion on a cover glass and spread in a thin film. Allow the film to air dry. Invert the cover glass (bacteria film down) and pass it through the flame of a Bunsen burner three times. This kills the bacteria and fixes them to the cover glass (the slime layer surrounding the cells sticks to the glass). Flood the smear with crystal violet (or other simple stain) and allow to stain two to three minutes. The staining time will vary with the stain you use and the organism you are staining. Wash the smear in tap water until no more stain washes out. Air dry or blot all but the surface of the smear and mount on a slide for examination with your microscope.

Examine all areas of the film with low power. Select a portion of the smear in which the bacteria are well distributed and sufficiently separated to see individual cells. Examine this portion under high power. You should be able to see the cells clearly and determine their shape and grouping with standard high-power magnification (430 or 440×). If an oil-immersion microscope is available (970 or 1,000×), you will be able to see the cells more clearly.

In the space below, draw several cells as you see them and classify them as to type (streptococcus, staphylococcus, bacillus, streptobacillus, etc.). If you used a colony in making your smear, the bacteria should be all of one type. In the bean infusion, several types should be present.

Bacteria stained with a simple stain

Section 2 *Gram's differential staining technique.* Gram's differential stain is perhaps the most widely used stain in bacteriology. Nearly all bacteria are classified as Gram-positive or Gram-negative by their reaction to this stain. The procedure involves the use of two stains. The initial stain (you will use crystal violet) stains all bacteria. Other reagents used in the procedure cause some bacteria to retain this initial stain and remain violet in color. These organisms are classified as *Gram-positive.* The procedure also makes use of 95% alcohol to decolorize certain bacteria. These organisms lose their violet color and are stained red with a counter stain, carbol fuchsin or safranin. We classify them as *Gram-negative.* The determination of bacteria as Gram-positive or Gram-negative is important, not only in classification but in determining many other properties, including their sensitivity to various antibiotics in the treatment of infectious diseases.

To make a Gram's stain preparation of a bacteria film, transfer bacteria from a colony in an agar slant or Petri-dish culture to a drop of distilled water on a cover glass, using a flamed inoculating loop as you did in using a simple stain. Air dry the film and kill and fix in a flame. Flood the film with crystal violet. Add three or four drops of 5% sodium bicarbonate solution and allow to stand for two or three minutes. Quickly, rinse in water until no more stain washes out of the film. (This removes the excess stain.) Flood the stained film with Gram's iodine for one to two minutes. (The iodine fixes the stain in Gram-positive bacteria.) Rinse in water. Using a dropping pipette, rinse the film with 95% alcohol until no more stain runs out. This decolorizing usually requires from 30 seconds to one minute. Flood the film with carbol fuchsin or safranin (as a counterstain) for 30 seconds to one minute. Rinse in water to remove the excess stain, air dry or blot the cover glass, and examine under high power. (a) What color are your bacteria? .... (b) Would you classify them as Gram-positive or Gram-negative? .... (c) Did all of the bacteria in your film produce the same staining reaction? .... If other members of your class made Gram's stain preparations, examine other films to see both Gram-positive and Gram-negative organisms. (d) Determine the ratio of Gram-positive to Gram-negative bacteria as determined by all members of the class. ....

Section 3 *Spore staining.* Spore staining is more difficult than either simple or Gram's differential staining. It involves the use of two stains—one which stains the spore and another which stains the vegetative cell a contrasting color. The stains you will use should color the spores red and the other cell structures blue. Color contrasts in spore staining results from differences in the permeability of the walls of spores and bacterial cells to stains. A spore has a thick, protective wall which is difficult to penetrate with a stain. However, having absorbed a stain a spore retains the color as it is washed out of the remainder of the cell with a decolorizing reagent. The cell is then counterstained with another color.

Use a bean infusion or a nutrient-broth culture of a known spore-forming organism for making your film on a cover glass. Use the same technique as in making a simple stain film. Flood the cover glass with carbol fuchsin. Heat the flooded film *gently* over a Bunsen flame until vapor rises. Steam for five minutes. (CAUTION: *Watch the cover glass carefully; do not allow the carbol fuchsin to dry.*) Decolorize with 5% acetic acid until the film becomes light pink. Stain with methylene blue for three to four minutes. Wash with water until all excess stain is removed. Air dry or blot the cover glass, mount on a slide and examine with low power and high power. (e) Are spores present in certain of the cells? .... (f) If so, describe the appearance of the spores. ....

(g) Describe the color of the cells. .... (h) Are spores which have escaped from cells present? .... (i) What shape are the cells of your spore-forming organisms? ....

Dilution and Plating-out Procedures 16-5

Purpose To become familiar with the methods and techniques involved in preparing and plating out a dilution series in a bacteriological analysis.

Dilution and plating-out procedures are widely used in determining the precise bacterial content in a sample of water, milk, and other food products. The bacterial population of these substances is an important index of pollution, infection, contamination during handling and processing, and spoilage. By preparing a dilution series of a measured quantity of the sample to be tested, the bacteriologist can reduce the bacterial population to lesser and lesser amounts. Having determined the number of bacteria in a known dilution, he can calculate the number of bacteria present in a measured quantity of the original sample with reasonable accuracy.

A Part 1 DEVELOPING SKILL IN THE USE OF PIPETTES. Since the preparation of a dilution series in a bacteriological analysis involves extreme accuracy in using calibrated glassware and in transferring measured quantities of liquids in pipettes, skill in these techniques is essential. Before preparing the dilution series of a water sample in Part 2, you will need practice in filling calibrated dilution bottles and in transferring water with 11-ml and 1-ml pipettes.

Materials. Two 160-ml APHA milk dilution bottles graduated at 99 ml, with screw caps or Escher rubber stoppers or 250-ml Erlenmeyer flasks and a graduated cylinder to measure 99 ml; 11-ml pipette; 1-ml pipette.

Procedure and observations. Since this Part is for practice only, the dilution bottles and pipettes need not be sterilized. However, it is important that you practice sterile techniques which will be necessary in work to follow. Avoid touching the rims or stoppers of the dilution bottles and the lower portions of the pipettes, which will be inserted into liquids.

Fill one dilution bottle to the 99-ml graduation with tap water. Hold the bottle at eye level to be sure that the water surface is exactly on the 99-ml graduation. If you are using an Erlenmeyer flask, measure exactly 99 ml of water in a graduated cylinder and pour it into the flask. Use the second bottle or flask to receive water as you practice transferring with the pipettes. Examine an 11-ml pipette and note the graduation at which the pipette will deliver exactly 11 ml of liquid. Hold the pipette near the mouthpiece with light pressure and with your forefinger raised in position to cover the upper end. Take the dilution bottle containing the water in your left hand and insert the pipette. Angle the bottle and pipette toward your mouth. *Slowly,* draw water into the pipette a short distance above the 11-ml graduation. As you remove the pipette from your mouth, place your forefinger over the upper end. This holds the column of water you drew into the pipette. Raise the bottle and pipette until the graduation is at eye level. Notice that the surface of the water column in the pipette is lens-shaped. This is called a *meniscus.* Release your finger pressure on the pipette, allowing water to flow from the tip a drop at a time. Stop the flow by pressure when the base of the meniscus is exactly on the graduation. Holding the pipette firmly, transfer the water to the second dilution bottle. Release your finger, allowing water to flow from the pipette. You have transferred exactly 11 ml of water. Notice that a small amount of water remains in the tip of the pipette. This water should not be blown out, since the pipette is calibrated *to deliver* 11 ml with this amount remaining.

Repeat this process several times until you can use the pipette and transfer 11 ml of water accurately. Then repeat the process with the 1-ml pipette. This pipette is more difficult to use because it fills and empties more rapidly. Make numerous practice transfers of 1-ml quantities of water. Do not attempt Part 2 until you have developed skill in using pipettes.

A Part 2 **PLATING OUT A WATER SAMPLE.** In this Part you will conduct a "trial analysis" of a water sample. This sample you use should have a relatively high bacterial content. A sample from a stream, a drainage ditch, a shallow well, or cistern is ideal. You will determine the number of bacteria present in 1 ml of the sample. Since you cannot see all of the bacteria present in this quantity of water, it will be necessary to grow the organisms in cultures, where each organism will form a visible colony after 48 hours of incubation. Each colony will, then, represent a bacterium present in the water. What if the number of bacteria present in 1 ml of water is many thousand? This is far too many colonies to count. This is the reason for making dilutions. Somewhere in the series, you should find a plate with a number of colonies you can count. By knowing the amount of the dilution, you can calculate the number of bacteria present in 1 ml of the sample.

Materials. Water sample; sterile screw cap or stoppered bottle for collecting water sample; six 160-ml APHA milk dilution bottles, graduated at 99 ml (or six 250-ml Erlenmeyer flasks and graduated cylinder for measuring 99 ml); seven 1-ml pipettes; six 11-ml pipettes; seven Petri dishes (100 mm); pipette can; hot-air oven; autoclave or pressure cooker; glass-marking pencil; incubator; refrigerator; colony counter with hand tally (if available); nutrient agar.

Procedure and observations. Section 1 *Preparing the materials.* Collect the water sample in a sterile bottle and store in the refrigerator until needed. Prepare nutrient agar, as outlined in Investigation 16-2 (150 ml per sample to be tested), sterilize in the autoclave or pressure cooker and store in media bottles until needed. (The media may be sterilized with the dilution bottles.) Store the sterile nutrient agar in the refrigerator until used.

Fill seven dilution bottles to the 99-ml graduation and screw on the caps or insert the rubber stoppers loosely. Sterilize in the autoclave or pressure cooker at 15 lbs for 15 minutes. After sterilization and cooling, use the seventh bottle to replace any water lost from the other six during sterilization. If 250-ml Erlenmeyer flasks are used, mark the 99-ml level on the flask with a glass-marking pencil. Replace water lost to this line, if necessary. Use rubber stoppers or cotton plugs to close flasks.

Sterilize seven 1-ml and six 11-ml pipettes (for each sample) in pipette cans in an oven at 350°F for two hours. If pipette cans or other metal containers are not available, wrap the sterile pipettes in clean paper as soon as they have cooled in the oven. Sterilize seven Petri dishes (for each sample) with the pipettes.

Section 2 *Plating out a dilution series.* Before starting your dilution series, be sure that the dilution bottles or flasks, pipettes, and Petri dishes have cooled to room temperature. The nutrient agar must be liquified (by reheating to boiling, if necessary) and cooled in a pan of water or a water bath to 45°C for pouring.

When you are ready to start your dilution series, mark the water sample 0, using a glass-marking pencil. Mark six dilution bottles 1:10, 1:100, 1:1,000; 1:10,000; 1:100,000, and 1:1,000,000. Place these bottles in a row, with the water sample on the left. Be sure that they are in proper order. Mark seven Petri dishes similarly and place each dish in front of its respective bottle, as shown in Fig. 16-5-a. Study the figure and follow each step carefully as you proceed through the dilution series.

Open the water sample and transfer 1 ml to the 0 plate (Petri dish), using a sterile 1-ml pipette. Lay the pipette aside after use. With an 11-ml sterile pipette, transfer exactly 11 ml of water to the 1:10 dilution bottle. Tighten the cap or stopper and shake vigorously 50 times. This bottle now contains a 1:10 dilution of the water sample (11 ml of sample and 99 ml sterile

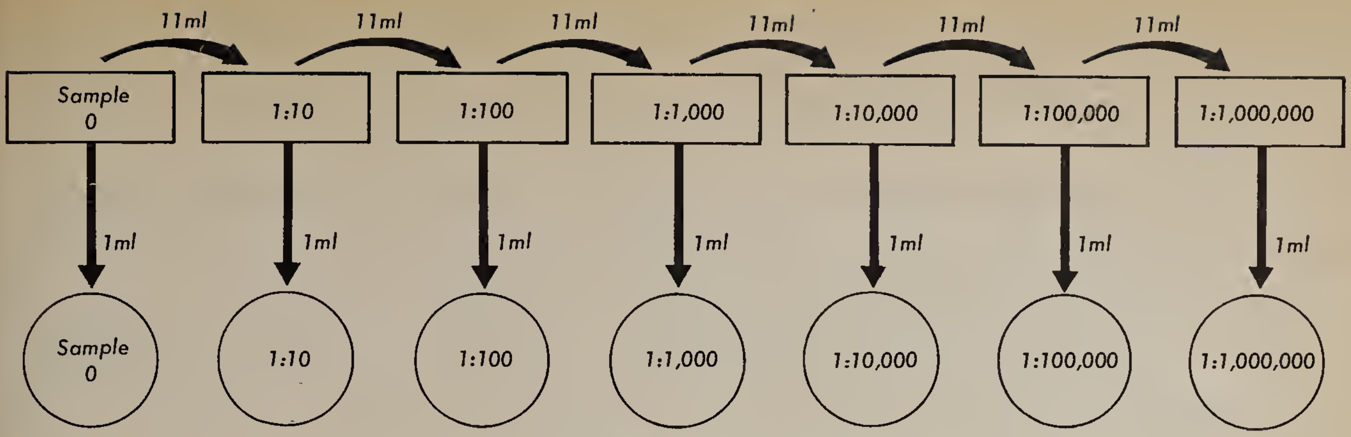


Fig. 16-5-a Diagram of a dilution series

water). With a sterile pipette, transfer 1 ml of this dilution to the 1:10 plate. Continue the series through all of the dilutions to 1:1,000,000.

When the dilutions are completed, pour the plates with sterile nutrient agar. Pour each plate to a depth of about 5 mm (one-fourth inch). Before the medium gels, agitate the plates to diffuse the inoculum (water) through the medium, using care not to splash the cover. When the plates have cooled and gelled, invert them, and incubate for 48 hours at 37°C (98°F).

Section 3 *Plate counts and determination.* After 48 hours, each bacterium will have produced a visible colony. Arrange the plates in the order of your dilutions. You should notice a decrease in the number of colonies as you proceed from the sample to each higher dilution. If your dilutions are extremely accurate, each plate should contain one tenth the number of colonies present in the dilution from which it was made. Fig. 16-5-b shows theoretical counts in a dilution series. A perfect series such as this could never be obtained and serves here only as an illustration.

Plate	Count
Sample-0	6,000,000
1:10	600,000
1:100	60,000
1:1,000	6,000
1:10,000	600
1:100,000	60
1:1,000,000	6
Bacteria count per ml	6,000,000

Fig. 16-5-b Plate counts in a dilution series

Plate	Count
Sample-0	....
1:10	....
1:100	....
1:1,000	....
1:10,000	....
1:100,000	....
1:1,000,000	....
Bacteria count per ml	....

Fig. 16-5-c Bacteria count determination in a sample of .... water

In the dilution series shown in Fig. 16-5-b the 0, 1:10, 1:100, and 1:1,000 plates contain too great a number of colonies to be counted. The bacteriologist would mark these plates *TNTC* (too numerous to count). The 1:10,000 plate, with 600 colonies, might be estimated from a partial count (one fourth or one half of the plate). The 1:100,000 and 1:1,000,000 dilution plates can be counted. However, a count should be based on a plate with 30 to 300 colonies. Thus, the 1:100,000 plate with 60 colonies should be used in the count determination. To determine the bacteria count in 1 ml of the original sample, multiply the plate count by the amount of the dilution (in this case, 60 × 100,000).

Examine each plate in your dilution series. Count the colonies where possible, and record the count in Fig. 16-5-c. A colony counter is ideal for making plate counts since it illuminates the plate and provides guide lines for systematic counting or counting a portion of a plate. A hand tally is valuable in registering the colony count. If a colony counter is not available, it may be necessary to divide the plate into sections with parallel lines drawn on the bottom of the Petri dish with a glass-marking pencil. This will aid greatly in counting and will avoid confusion in keeping track of areas counted.

If possible, determine the bacteria count per ml of the original sample from a plate with 30-300 colonies.

(a) Would a dilution series have been necessary to determine a bacteria count per ml in the sample you used? .... (b) Explain. ....

....

(c) Explain why a plate with more than 300 colonies should not be used in determining a bacteria count. ....

....

....

The higher the dilution necessary to obtain a plate count, the greater the possibility of error in determining the bacteria count in the sample. (d) Why would this be true? ....

....

Bacteriological Analysis of Milk Products

16-6

Purpose To determine the bacterial content of various milk products.

Milk, while it is one of our most nutritious foods, is also a potential source of infection since it is an excellent culture medium for many bacteria. For this reason, the dairy industry must maintain high standards of sanitation in handling and processing the milk supply. Milk normally contains a large number of harmless milk-souring organisms. In fact, in processing certain milk products such as buttermilk and cottage cheese, bacteria are purposely added as "starters" which are essential in producing the product. However, the number of bacteria in whole milk, skimmed milk, chocolate milk, half and half milk, and cream should be held to the lowest number possible to delay souring and spoilage. Proper sanitation, handling, and refrigeration are necessary, also, in preventing the growth of infectious organisms that may be introduced into milk during processing.

B Part 1 DETERMINATION OF BACTERIAL CONTENT OF VARIOUS MILK PRODUCTS. Milk taken from a healthy cow should be sterile. However, with each exposure to the air or handling in containers, the bacterial content increases. Pasteurization reduces this count greatly, and proper refrigeration will retard the growth of the remaining bacteria. Even under the best possible handling conditions, however, milk products contain large numbers of bacteria which multiply rapidly as the milk ages.

Materials. Milk products, including plain pasteurized whole milk (if available), homogenized whole milk, half and half milk, skimmed milk, chocolate milk, cream, buttermilk, and ice cream; Petri dishes (five per sample); dilution bottles (six per sample); 1-ml pipettes (five per sample); 11-ml pipettes (six per sample); 8-oz bottle (for ice cream); autoclave or pressure cooker; pan or water bath for cooling medium to souring temperature; centigrade thermometer; hot-air oven; pipette cans; colony counter (if available); plate-count agar (or other medium recommended for milk organisms).

Procedure and observations. Prepare sufficient plate count agar to pour five Petri dishes per sample (120 ml per sample) as directed on the bottle. Put the prepared medium in bottles, sterilize at 15 lbs for 15 minutes in an autoclave or pressure cooker, cool, and refrigerate until used. Prepare dilution bottles as outlined in Investigation 16-5 and sterilize with the medium (six per sample). Each sample will require seven 1-ml pipettes and six 11-ml pipettes. Sterilize in an oven in pipette cans for two hours at 350°F. Sterilize the Petri dishes to be used (five per sample) with the pipettes.

Number all milk product samples and a corresponding set of plates to include dilutions of 1:100 through 1:1,000,000. The 0 and 1:10 plates will be omitted in the series since the milk clouds the agar and makes counting difficult.

Using the dilution and plating-out techniques outlined in Investigation 16-5, prepare dilutions of all samples from 0 to 1:1,000,000. Inoculate plates with 1-ml portions of the 1:100 through 1:1,000,000 dilutions of each sample. It will be necessary to melt the ice cream sample in a sterile bottle before making the dilutions. Pour all plates with plate-count agar, previously liquified by boiling (or removed from the sterilizer while hot) and cooled to 45°C. Before the agar gels in the plates, agitate to diffuse the inoculum through the medium uniformly. Allow the plates to gel. Incubate at 37°C for 48 hours.

After incubation, arrange the plates for each sample in order of dilutions. Count the colonies on all plates where it is possible and record your results in the table provided. Count the colonies on as many plates as possible in each series. Where there is a large number of colonies, count ½ or ¼ of the plate and multiply the count by the portion counted. Indicate plates with colonies too numerous to count as *TNTC* in the table. Calculate the number of bacteria in 1 ml of the sample from a plate in the series containing 30-300 colonies, if possible. This count should correlate with reasonable accuracy with a count determined from the next lower and next higher dilutions. Record the number of bacteria per ml for each sample in the table provided. Construct a bar graph below the table, showing the bacteria count per ml of each sample. Use a different color for each bar and identify it with a color key.

Milk Product	*Plate*	*Plate Count*	*Milk Product*	*Plate*	*Plate Count*
Sample #1	1:100 1:1,000 1:10,000 1:100,000 1:1,000,000		Sample #4	1:100 1:1,000 1:10,000 1:100,000 1:1,000,000	
Bacteria count/ml			Bacteria count/ml		
Sample #2	1:100 1:1,000 1:10,000 1:100,000 1:1,000,000		Sample #5	1:100 1:1,000 1:10,000 1:100,000 1:1,000,000	
Bacteria count/ml			Bacteria count/ml		
Sample #3	1:100 1:1,000 1:10,000 1:100,000 1:1,000,000		Sample #6	1:100 1:1,000 1:10,000 1:100,000 1:1,000,000	
Bacteria count/ml			Bacteria count/ml		

Bar graph of bacterial counts per ml

Interpretation. On the basis of your data, account for the variation in bacterial count per milliliter of the milk products you used. Consider such factors as age of the milk or milk product, amount of handling and processing, ingredients present or added in processing, inoculation with "starter" organisms, and other significant conditions.

Effect of Temperature on the Growth of Bacteria in Milk

16-7

Purpose To determine the relation of temperature to the rate of growth and reproduction of bacteria in milk; to demonstrate the effectiveness of pasteurization methods and boiling in reducing the number of bacteria in milk.

As you are aware, milk may be held several days to a week without souring in a refrigerator, but will sour in a few hours at summer heat. The rate at which souring and spoilage occur is related directly to the metabolic activity and the rate of increase in the number of bacteria present. Refrigeration at a temperature of 40-45°F reduces bacterial activity and, therefore, delays souring and other chemical changes resulting from bacterial action. On the other hand, heating milk to temperatures above 140°F for a short time destroys many of the bacteria present. This is the reason for pasteurization.

B Part 1 EFFECT OF TEMPERATURE ON THE RATE OF BACTERIAL INCREASE IN MILK. The relation of temperature to the rate of increase in the bacterial content of milk and resulting souring and spoilage may be shown by determining the number of bacteria present during intervals at which milk is held at various controlled temperatures. The dilution and plating-out techniques described in Investigation 16-5 will be used in determining bacteria counts in milk in this Part.

Materials. One quart of fresh pasteurized homogenized milk (to be used in preparing three samples for seven bacteria-count determinations over a period of three days); three 8-oz sterile bottles (for milk samples); Petri dishes (five per determination); dilution bottles (six per determination); 1-ml pipettes (five per determination); 11-ml pipettes (six per determination); pipette cans; incubator; refrigerator; autoclave or pressure cooker; sterilizing oven; pan or water bath (for cooling liquified agar to 45°C for pouring); centigrade thermometer; plate-count agar; media bottles.

Procedure and observations. Bacterial counts to be determined in this Part will require a total of seven sets of dilutions, prepared on three consecutive days. Each set of five plates will require 120 ml of plate-count agar.

Prepare 1,000 ml of plate count agar (allowing some extra medium) as directed on the media bottle. Bottle the prepared media, sterilize in the autoclave or pressure cooker at 15 lbs for 15 minutes, and store in the refrigerator until needed. Fill the dilution bottle (six per count determination to be made) to 99 ml. Allow one extra bottle for adding water lost in sterilization. Sterilize the dilution bottles and three 8-oz bottles for milk samples with the media. Sterilize 1-ml pipettes (five per count determination) and 11-ml pipettes (six per count determination) in pipette cans in the oven for two hours at 350°F.

Obtain a bottle of fresh pasteurized homogenized whole milk, delivered from the dairy that morning, if possible. Pour part of the milk into each of three sterile 8-oz bottles designated as samples *1, 2,* and 3. Put sample 1 in the refrigerator, 2 in a cupboard at room temperature, and 3 in the incubator at 35-37°C.

The remainder of the fresh milk will be used to determine the bacterial count in the milk used in all samples at the start of the Investigation. Prepare a dilution series from 0 through 1:1,000,000, using the procedure outlined in Investigation 16-5. Prepare plates of the dilutions from 1:100 through 1:1,000,000. Pour the plates with plate count agar, cooled to 45°C for pouring, and incubate at 35-37°F for 48 hours. Examine all plates in the series and count as many as possible. Record your results in the "Count start" column of the table, repeating your data for all three samples, since all are the same milk at the start. Determine the bacteria count per ml from one of the plates in the series (30-300 colonies if possible) and record this as the "Bacteria per ml-Start" for all three samples.

On the second day (24 hours), prepare dilutions and plates of samples 1, 2, and 3. Make plates of the 1:100 through the 1:1,000,000 dilutions. Incubate the plates at 35-37°F for 48 hours. Make notes of any changes in the physical appearance of the milk in the three samples and any odors you can detect. After incubation, count as many plates as possible, recording those with colonies too numerous to count as *TNTC*. Record these plate counts in the column marked "Plate count-24 hours" in the table. Determine the plate count per ml for each sample from the colony count on one plate in the series (30-300 colonies if possible). Record these counts in the column of the table marked "Bacteria per ml-24 hrs."

On the third day (48 hours), repeat the dilutions and plating out of the three samples. Incubate the plates at 35-37°F for 48 hours. Count the plates, determine the bacteria per ml, and record these data in the 48-hour columns in the table.

Sample source	*Plate*	*Count—start*	*Bacteria per ml—start*	*Plate count—24 hrs*	*Bacteria per ml—24 hrs*	*Plate count—48 hrs*	*Bacteria per ml—48 hrs*
1 Refrigerated	1:100 1:1,000 1:10,000 1:100,000 1:1,000,000						
2 Room temperature	1:100 1:1,000 1:10,000 1:100,000 1:1,000,000						
3 Incubator	1:100 1:1,000 1:10,000 1:100,000 1:1,000,000						

You may wish to show the increase in bacterial content in the three samples with a line graph, prepared on a sheet of graph paper. Space the time intervals (0, 24 hrs, 48 hrs) along the horizontal axis and indicate the bacteria counts along the vertical axis. Use different colors for drawing the lines showing count increases in the three samples and identify each with a color key.

The increase in numbers of bacteria, especially in the room temperature and incubated milk, was accompanied by physical and chemical changes. Describe any changes in appearance or odors after 24 hours (a) in the refrigerated milk. ....

.... (b) in the room temperature milk. ....

.... (c) in the incubated milk. ....

....

Describe changes and odors after 48 hours (d) in the refrigerated milk. ....

....

(e) in the room temperature milk. ....

....

(f) in the incubated milk. ....

Interpretation. On the basis of your data and observations, discuss the relation of temperature to the rate of increase in the bacterial content of milk. ....

....

....

B Part 2 EFFECT OF PASTURIZATION AND BOILING ON THE BACTERIAL CONTENT OF RAW MILK. In his studies of fermentation in wines, Louis Pasteur discovered that heating a fermenting fruit juice destroyed large numbers of bacteria and delayed or prevented further chemical changes during aging of the wine in closed containers. Later, the process known as pasteurization was applied to milk and other products.

Two methods are used today in the pasteurization of milk. In the *holder method,* widely used in the dairy industry, milk is heated to 143-145°F (60-62°C) and held at this temperature for 30 minutes. The milk is then cooled quickly to prevent the growth of spore-forming, protein-splitting (proteolytic) bacteria which survive the heat. In the *flash method* of pasteurization, a thin film of milk is heated rapidly to a temperature of 160-162°F (71-72°C) for 15 seconds, after which it is cooled quickly. Both pasteurization methods are designed to destroy tuberculosis organisms as well as other pathogens. Many other bacteria are destroyed in the process, thus delaying souring and other spoilage.

Boiling destroys nearly all of the bacteria in milk with the exception of spores. While boiling may render the milk more nearly sterile, the high temperature causes coagulation and produces undesirable flavors.

Materials. One quart of unpasteurized raw milk (obtained from a farm or a dairy); four 500-ml Erlenmeyer flasks; cotton for plugging flasks; Petri dishes (seven per sample); 160-ml APHA dilution bottles, filled to 99 ml with water; 11-ml pipettes (six per sample); 1-ml pipettes (five per sample); pipette cans; pan (at least 6 in. deep); large rubber solid stoppers (for supporting flasks in pan of water); hot plate, or burner and tripod; incubator, autoclave or pressure cooker; pan or water bath (for cooling medium for pouring); thermometer (C or F); colony counter; plate-count agar (150 ml per sample); glass-marking pencil; cracked ice.

Procedure and observations. Prepare 600 ml (150 ml per sample) of plate-count agar, sterilize in media bottles, and store in the refrigerator until used. Sterilize the prepared dilution bottles and four 500-ml Erlenmeyer flasks plugged with cotton in the autoclave or pressure cooker with the media. The Petri dishes and pipettes should be sterilized in a hot-air oven, as directed in Investigation 16-5.

Obtain a quart of raw unpasteurized milk and divide it equally in the four sterile Erlenmeyer flasks. Mark the flasks *samples 1, 2, 3,* and *4.* Immediately, make a dilution series of sample 1 (raw, untreated milk) from 0 through 1:1,000,000. Plate-out 1 ml portions of the 1:100 through the 1:1,000,000 dilutions.

Fill a pan with tap water and place it on the hot plate. A second pan should be filled with cold water and cracked ice. Put sample 2 in the pan of water with the flask resting on rubber stoppers to raise it from the bottom of the pan. This will prevent scorching the milk. Put a thermometer in the water. The level of water in the pan must be above that of the milk in the flask. Heat the water to a temperature of 143-145°F (60-62°C). When this temperature is reached, reduce the heat to hold the milk within 1 or 2 degrees for exactly 30 minutes. After heating, cool the milk in ice water. Prepare a dilution series of this milk as soon as it cools, repeating the procedure used for sample 1.

Starting with water at room temperature, heat the milk in sample 3 to 160-162°F (71-72°C). Watch the thermometer very closely. Remove the flask after 15 seconds at this pasteurization temperature. Cool the milk in ice water and prepare the dilutions and plates as in the other samples. (This method will not duplicate the commercial flash pasteurization procedure, since the milk was heated to pasteurization temperature more gradually, thus increasing the duration of the thermal treatment.)

Using the same method, heat the milk in sample 4 to boiling. Cool quickly and prepare a dilution series and set of plates. Pour all of the plates with plate-count agar, previously liquified and cooled to 45°C. Incubate the cultures at 35-37°C for 48 hours.

Immediately after plating out the four samples, taste the milk in samples 2, 3, and 4. Examine these samples and note any change in the physical appearance of the milk. Record your observations and any flavor you detect in the table provided.

After incubation, count all plates in each series. Using a colony counter, if available, count as many plates in each series as possible. Record your plate counts in the table. Determine the bacteria count per ml for each sample from a plate with 30-300 colonies, if possible. Otherwise, use the next higher or next lower dilution for your calculation. Record these counts in the table.

Milk sample	*Plate*	*Plate count*	*Bacteria per ml*	*Flavor*	*Physical change*
Raw milk	1:100 1:1,000 1:10,000 1:100,000 1:1,000,000				
Pasteurized Holder method	1:100 1:1,000 1:10,000 1:100,000 1:1,000,000				
Pasteurized Flash method	1:100 1:1,000 1:10,000 1:100,000 1:1,000,000				
Boiled	1:100 1:1,000 1:10,000 1:100,000 1:1,000,000				

Interpretation. On the basis of your data, explain the purpose and the effectiveness of pasteurization of milk. ....

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Purpose To determine the bacterial content and possible presence of coliform organisms in various water supplies.

Bacteriological analysis of our water supply is vital in preventing epidemics that may result from water pollution. Sewage contamination of the water supply is a constant danger, especially in heavily populated areas. Is water in a well safe to drink, or is it contaminated with intestinal pathogens such as typhoid fever, paratyphoid, dysentery, or cholera? Are our lakes and streams safe for swimming, or menaces to the health of the community? These are questions only the bacteriologist can answer.

B Part 1 BACTERIOLOGICAL ANALYSIS OF WATER SUPPLIES. When a bacteriologist analyzes water from a well, a cistern, a spring, or some other source from which water is being used, he is interested primarily in two things. What is the total bacteria count per ml of the water? This total count, based on a plate count, indicates the introduction of bacteria from soil, organic matter, and other common sources. However, he is most concerned with the presence of bacteria that normally live in the human intestine. He refers to these as *coliform* bacteria. The presence of coliform organisms is evidence of sewage pollution and the possible presence of intestinal pathogens.

In determining the total bacteria count in the water samples, you will prepare a dilution series and use the plating-out technique you used in milk analysis. However, in the coliform analysis, you will make use of special media. These media show special reactions and growth characteristics of coliform organisms.

Materials: Wide-mouth bottles with ground glass stoppers or caps, 4-8 oz (for collecting water samples); Petri dishes (seven per sample); dilution bottles (six per sample); 1-ml pipettes (seven per sample); 11-ml pipettes (six per sample); pipette containers; 6-in. culture tubes and APHA $1\frac{15}{16}$-in. tubes for preparing fermentation tubes (five each per sample); tube rack or basket; cotton for plugs; inoculating needle; autoclave or pressure cooker; hot-air oven; ring stand, funnel, rubber tube and tubing clamp (for filling fermentation tubes); colony counter; nutrient agar (150 ml per sample); eosin methylene blue agar or Endo agar plates (for coliform test); lactose broth.

Procedure and observations. Section 1 *Preparation of media.* Prepare the nutrient agar as directed in Investigation 16-2, sterilize in media bottles and store in the refrigerator until used. Prepare sufficient eosin methylene blue agar or Endo agar to allow about 100 ml for possible use for each sample. After sterilization, this media may be stored in bottles or poured into sterile Petri dishes (15-20 ml per dish). Refrigerate this medium until used. Prepare lactose broth according to the directions on the bottle (50 ml per water sample to be tested). After dissolving the lactose broth in distilled water, pour the broth into a funnel equipped with a rubber delivery tube and clamp, supported on a ring stand. To prepare a fermentation tube, drop an APHA $1\frac{15}{16}$-in. culture tube, open end down, into a 6-in. culture tube. Fill each fermentation tube half full of lactose broth. Plug with cotton and place in a tube rack or basket for sterilizing. Prepare five lactose broth fermentation tubes for each water sample. Sterilize the fermentation tubes in an autoclave or pressure cooker at 15 pounds for 15 minutes. Air should be forced out of the small tubes during sterilization and cooling.

Prepare and sterilize the dilution bottles, pipettes, and Petri dishes as directed in Investigation 16-5. The ground glass stopper or screw-cap bottles for collecting water samples may be boiled for 10 minutes or sterilized in an autoclave or pressure cooker.

Section 2 *Plate count and coliform determinations.* Collect water from five or more water supplies, including such sources as a shallow (dug) well, deep (driven) well, cistern, spring, and city water supply. Be sure to identify each of your samples.

Liquify nutrient agar for pouring plates as directed in Investigation 16-2. Mark seven Petri dishes for each sample, indicating the sample number, dilution factor (0 through 1:1,000,000) and date. Mark six dilution bottles with dilution factors 1:10 through 1:1,000,000 for each sample. Place five lactose broth fermentation tubes in a row in a tube rack for each sample. Each set of five tubes need be identified only by sample number and date.

Using a sterile 1-ml pipette, transfer 1 ml of water from sample 1 to each of five lactose broth fermentation tubes and to the 0 dilution plate. Continue the dilution series and 1-ml plate inoculations through the 1:1,000,000 dilution. Repeat the procedure for all other samples, inoculating five lactose broth fermentation tubes for each sample and preparing a set of seven plates from the dilution series. Pour all plates with nutrient agar at 45°C, diffuse the inoculum into the media by agitating the plate, and allow to gel. Incubate the lactose broth tubes and plates at 35-37°C.

After 24 hours, examine the lactose broth fermentation tubes. If gas is present in the small tube (the tube may float to the surface) mark a plus (+) in the appropriate column of the table. If no gas is present, mark a minus (−).

The formation of gas in lactose broth in 24 hours is considered a *positive presumptive test* for coliform organisms. A second test is used for each tube showing gas. Liquify eosin methylene blue agar or Endo agar and pour about 20 ml into sterile Petri dishes for streaking (one for each positive broth fermentation tube). Identify the plates by sample number corresponding to the fermentation tubes. Using an inoculating needle, streak an eosin methylene blue agar or Endo agar plate with each positive tube. Return the negative lactose broth fermentation tubes and streaked eosin methylene blue agar or Endo agar plates to the incubator. After streaking, you may discard the positive lactose broth tubes.

After a second 24-hour incubation period, remove all plates and tubes from the incubator. Count as many plates as you can in the dilution series with a colony counter and record the counts in the table. Indicate plates with colonies too numerous to count as *TNTC*. Calculate the bacteria count per ml of water from a plate showing 30-300 colonies, if possible. Record this count in the table.

Examine the remaining lactose broth fermentation tubes. Record them as positive (+) or negative (−) after 48 hours. The production of gas within 48 hours but not within 24 hours is considered a doubtful presumptive coliform test. However, these tubes should be streaked on eosin methylene blue agar or Endo agar, incubated, and examined after 24 hours.

Examine the eosin methylene blue agar or Endo agar plates streaked from fermentation tubes showing gas in 24 hours. Characteristics of the growth on these differential media are used to distinguish coliform bacteria (*Escherichia coli*) from organisms of nonfecal or intestinal origin which ferment lactose. *Escherichia coli* forms a growth with a flat surface and a greenish metallic sheen on eosin methylene blue agar. On the other hand, noncoliform lactose fermenters (including *Aerobacter aerogenes*) form a light pink, mucoid (slimy) growth. The growth of *Escherichia coli* on Endo agar is deep red, with red coloration of the surrounding media. Noncoliform organisms form a pink, mucoid growth on Endo agar. Indicate plates with coliform organisms as positive (+) for the confirming test in the table and those with noncoliform organisms as negative (−).

In some cases, certain coliform organisms fail to develop characteristic colonies on eosin methylene blue agar or Endo agar, or may form typical colonies. For this reason, a negative confirming test for coliform organisms cannot be considered conclusive proof of the absence of coliform organisms in water. A further and more complicated *completed test* may be used. This test is outlined in *Standard Methods for Water and Sewage Analysis* and in many bacteriology books.

Report your coliform analysis as the number of plates that were positive in the confirming test on eosin methylene blue agar or Endo agar.

Bacteria Count		*Coliform Analysis*		
Dilution	*Colony count*	*Presumptive test*		*Confirming test*
0		Lactose 24 hours	Lactose 48 hours	EMB or Endo agar 24 hours
1:10				
1:100				
1:1,000				
1:10,000				
1:100,000				
1:1,000,000				
Bacteria count/ml ____________		Coliform analysis ______________________________		

Interpretation. Using the data summarized in your table, discuss each sample as safe or unsafe to drink and the reasons for your decision. If possible, account for the plate counts in each sample and for the presence or absence of coliform organisms.

C Part 2 STREAM ANALYSIS

Problem. How does the total number of bacteria and coliform content per ml vary from one area of a stream to another?

Since the type of industry, homes, or use of the land along a stream determines the nature and extent of pollution of the water, you will find it interesting to analyze water from a series of collecting stations in a stream in your community. A stream survey is especially interesting where the stream flows through a city. Is sewage entering the stream from septic tanks or sewers? Is organic waste material which would increase the total count dumped into the stream along its course? A bacteriological analysis of a stream at various stations along its course may answer these and other questions.

Suggested materials. Petri dishes (seven per sample); 1-ml pipettes (seven per sample); 11-ml pipettes (six per sample); nutrient agar; lactose broth; eosin methylene blue agar or Endo agar; 6-in. culture tubes and $1^{15}/_{16}$-in. tube for fermentation tubes (five per culture); collecting bottles for water samples.

Suggested procedure. Collect several samples of water in sterile bottles from different stations. Collect from the mouth of the stream, if possible, and then at various intervals along the stream. Be sure to identify each of your samples. Use a field notebook to record the nature of the area. Is it heavily populated? Do the homes have septic tanks or sewers? Is industry located along the banks so that it is dumping materials into the river or stream? Is it in an area of farmland? Also note whether the water has run over a dam, whether the current is swift or still, and whether there is a rocky, sandy, or clay bottom to the stream. All samples should be tested without delay.

Use the same procedure for preparing media and running the tests as followed in Part 1 of this Investigation. Prepare a table similar to the one used in Part 1 for each sample. Then prepare a bar graph of the count per milliliter of each sample.

Analysis and conclusions. Your survey should help you to understand the problems involved in pollution. You should be able to draw some conclusions concerning the influence of surrounding areas on the bacterial count in the various areas of the stream.

Effect of Antiseptics on the Growth of Bacteria

17-1

Purpose To determine the effect of various antiseptics on the growth of bacteria and to determine the lethal concentration of certain antiseptics.

How effective are various commonly used antiseptics? Are they as effective as the advertising claims made for some of them? Are there differences in strength that determine the nature and area of use of various antiseptics?

B Part 1 THE INHIBITORY EFFECT OF VARIOUS ANTISEPTICS ON BACTERIA. In this Part you will determine the bactericidal action of various antiseptics against known test organisms by measuring zones of inhibition on inoculated plate cultures. You will compare the effectiveness of each antiseptic with that of a 5% phenol solution, which is used as a standard.

Materials. Common antiseptics (10 or more; you may wish to include mouthwashes); 5% phenol solution; filter paper; forceps; Petri dishes (one for each antiseptic and each organism to be tested); 250-ml Erlenmeyer flask (one for each test organism); nutrient agar; nutrient broth (250 ml for each test organism); cotton plugs for flasks; sterile 1-ml pipettes (one for each test organism); bulb to fit 1-ml pipette; paper punch (size to punch holes for notebook fillers unless prepared sterile paper disks are available); metric scale; stock cultures of *Escherichia coli, Bacillus subtilis, Proteus vulgaris,* and *Serratia marcescens* (if only two organisms are used, *Escherichia coli* and *Bacillus subtilis* are suggested); wax pencil or gummed labels.

Procedure and observations. Prepare four 250-ml Erlenmeyer flasks of sterile nutrient broth, sufficient nutrient agar to pour 40 Petri dishes (one for each antiseptic and test organism), sterile Petri dishes and two sterile 1-ml pipettes, as directed in Part 1 of Investigation 16-2.

Inoculate a nutrient broth culture with each of the test organisms. Incubate at 35-37°C for 48 hours.

Obtain a variety of antiseptics, including 5% phenol solution.

Using a sterile 1-ml pipette equipped with a bulb, transfer 1 ml of the *Bacillus subtilis* inoculum to each a set of sterile Petri dishes. Mark these dishes *set A* and number the antiseptics, using *1* for the 5% phenol solution. Mark these dishes *A-1, A-2,* etc. Using another serile pipette, prepare a similar set of culture dishes with the *Escherichia coli* inoculum, marking the culture dishes *B-1, B-2,* etc. Indicate the *Proteus vulgaris* inoculum as *set C* and *Serratia marcescens* as *set D.* Cool liquified nutrient agar to 45°C and pour all of the dishes, using care to diffuse the inoculum through the warm nutrient agar before it gels.

Using a notebook paper punch, punch small disks of filter paper (unless prepared disks are available). With forceps, dip three filter paper disks into the first antiseptic. Drain excess liquid from the disk so as not to drip antiseptic on the surface of the poured plate. Place one disk at the location of 12 o'clock on the face of a clock, one at four o'clock, and one at eight o'clock. Disks should be placed three-quarters of an inch from the rim of the Petri dish. Prepare similar plates for B-1, C-1, and D-1. Continue the process for each antiseptic and each of the test organisms. Incubate all plates for 48 hours at 35-37°C.

After incubation, examine the plates for evidence of a zone of inhibition around the disks. These will appear as clear areas and indicate destruction of bacteria or inhibition of growth. Invert the dish and lay a metric scale across each disk. Measure the width of the zone of inhibition on each side of the disk. Place the scale at right angles to the first measurements and obtain two more measurements. Average the four measurements for each of the three disks on each plate and record the data in the table provided.

Antiseptic	*Inhibition Zone*			
	Bacillus subtilis	*Escherichia coli*	*Proteus vulgaris*	*Serratia marcescens*

Compare the average zone measurement of each antiseptic with that of the zone around the 5% phenol disks for each organism. Discuss the relative effectiveness of each antiseptic in comparison with 5% phenol solution.

Interpretation. On the basis of your results, discuss variations in the bactericidal and inhibitory effects of the various antiseptics you used on various organisms and variations in the effectiveness of the same antiseptic on different organisms.

B Part 2 DETERMINATION OF A LETHAL CONCENTRATION OF ANTISEPTICS ON TEST ORGANISMS. The effectiveness of different antiseptics can be demonstrated by determining the concentration necessary to kill bacteria. This can be done by adding varying amounts of antiseptics to measured quantities of broth cultures of known bacteria.

Materials. Broth cultures of *Escherichia coli* and *Bacillus subtilis* (prepared in Part 1); two antiseptics (tincture of merthiolate 1:1,000, mercurochrome 2%, or tincture of iodine 2% are good); 44 sterile, plugged culture tubes (11 for each antiseptic and test organism used); 44 sterile Petri dishes (11 for each antiseptic and test organism used); 2 sterile 10-ml pipettes; bulb to fit 10-ml pipette; two dropping pipettes (medicine droppers; one for antiseptic); wax pencil or gummed labels; sterile nutrient agar (to pour 44 Petri dishes); inoculating loop; inoculating needle; concave slides and cover glasses (for hanging drops); microscope; test-tube rack.

Procedure and observations. Place four rows, 11 tubes each, of sterile, plugged culture tubes in a test-tube rack. Using a wax pencil or gummed labels, identify the rows as *A, B, C,* and *D.* Number the tubes in each row *1* through *11.*

Using a 10-ml sterile pipette equipped with a bulb, transfer 20 ml of the *Bacillus subtilis* broth inoculum prepared for Part 1 to each of the tubes in row A and row C. Using another sterile 10-ml pipette equipped with a bulb, transfer 20 ml of the *Escherichia coli* broth inoculum prepared for Part 1 to each of the tubes in row B and row D.

Various antiseptics may be used in this experiment (mercurochrome 2%, tincture of merthiolate 1:1,000, or tincture of iodine 2% are suggested). Using a dropping pipette, add 1 drop of the first antiseptic to tube 1 of set A; then, add 2 drops to tube 2, and continue increasing the number of drops to tube 10, which will receive 10 drops. Tube 11 will serve as a control. Repeat the process in the tubes in set B. Thus, the tubes in sets A and B will contain the same antiseptic but different organisms. Using the second antiseptic, prepare similar tubes in sets C and D. Incubate all of the tubes at 35-37°C for 48 hours.

After incubation, examine all of the tubes for evidence of living bacteria. This may be determined by the turbidity (cloudiness) of the broth, by microscopic examination, and by streaking the broth inoculum on nutrient agar plates.

Compare the turbidity of all tubes in each set with the control. Estimate the turbidity as *heavy* (*H*), *medium* (*M*), *light* (*L*), or *none* (*N*). Record your observations of all tubes in the table provided.

Using an inoculating loop, prepare hanging drops of all tubes and examine with a microscope for evidence of viability of bacteria in the broth. Indicate the presence of living organisms with a plus sign (+) and the absence of living organisms with a minus sign (−). Record your results in the table provided.

Pour 44 sterile Petri dishes, one for each tube, with sterile nutrient agar and allow the plates to gel. Mark the plates to correspond with the broth tubes. Using an inoculating needle, streak broth from each tube onto an agar plate, using a zigzag or serpentine streak. Incubate the plate cultures at 37°C for 48 hours. After incubation, examine all plates for evidence of growth of the organisms. Indicate growth with a plus sign (+) and lack of growth with a minus sign (−) in the table provided.

Set A—Action of ______________ on Bacillus subtilis											
Drops per 20-ml inoculum	1	2	3	4	5	6	7	8	9	10	Control
Turbidity											
Viability in broth											
Growth on nutrient agar											

Set B—Action of ______________ on Escherichia coli											
Drops per 20-ml inoculum	1	2	3	4	5	6	7	8	9	10	Control
Turbidity											
Viability in broth											
Growth on nutrient agar											

Set C—Action of ________________________ *on Bacillus subtilis*										
Drops per 20-ml inoculum	1	2	3	4	5	6	7	8	9	10	Control
Turbidity											
Viability in broth											
Growth on nutrient agar											

Set D—Action of ________________________ *on Escherichia coli*											
Drops per 20-ml inoculum	1	2	3	4	5	6	7	8	9	10	Control
Turbidity											
Viability in broth											
Growth on nutrient agar											

Interpretation. On the basis of results recorded in your summary table, determine as accurately as possible the lethal concentration, in drops per 20 ml, of the two antiseptics used on the two organisms tested. Account for possible variations in the results of turbidity determinations, results of viability tests, and results of the streak plates in various antiseptic concentrations. ....

Effect of Antibiotics on Bacteria

17-2

Purpose To determine the effect of certain antibiotics on the growth of bacteria; to study the combined action of antibiotics and metallic salts; and to search for antibiotics in the soil.

Many of you have probably been treated for an illness with one or more antibiotics. Do you know why a specific antibiotic is selected for a certain illness? Is one antibiotic more effective against certain organisms than others? Are some organisms resistant to antibiotics?

B Part 1 THE INHIBITORY EFFECT OF ANTIBIOTICS ON BACTERIA. Many investigators use the disk technique to determine whether bacteria are sensitive, moderately sensitive, or resistant to an antibiotic.

Materials. Stock cultures of *Bacillus subtilis, Escherichia coli, Serratia marcescens,* and *Proteus vulgaris;* 500-ml Erlenmeyer flask (1 per bacterium tested); six 8-oz media bottles; cotton; inoculating loop; pressure cooker or autoclave; dry air oven; Petri dishes (1 per bacterium and antibiotic to be tested); 1-ml pipettes (1 per organism to be tested); bulb to fit pipettes; Difco-sensitivity disks saturated with known quantities of antibiotics (penicillin, streptomycin, terramycin, chloromycetin, and aureomycin); nutrient agar; nutrient broth; distilled water.

Procedure and observations. Prepare nutrient agar and nutrient broth (250 ml for each bacterium tested) as directed on the bottle. Fill 8-oz media bottles with the agar and pour 250 ml of broth into individual Erlenmeyer flasks. Plug the openings of the flasks with cotton. Do not tighten the caps on the media bottles. Fill two 8-oz media bottles with distilled water. Sterilize the medium and water in a pressure cooker or autoclave for 15 minutes at 15 pounds. After sterilization, tighten the caps on the agar bottles and refrigerate until needed. Allow the nutrient broth to cool.

After the broth has cooled, you can prepare the inoculum (bacteria culture). With a sterile inoculating loop, transfer a sample of bacteria to the sterile broth. Replug the flask and shake it to distribute the bacteria. Prepare other flasks with the additional bacteria you wish to use for this Investigation. Incubate all flasks for 48 hours at 37°C. After 48 hours you may have a rich inoculum of each of the bacteria.

Identify each of the sterile Petri dishes with the name of the bacteria you are testing. Using a pipette bulb (not your mouth), transfer 1 ml of the inoculum to the appropriate Petri dish. Then pour 20 ml of the sterile nutrient agar which has been previously liquefied and cooled to 45°C into each Petri dish. Agitate the plates so as to diffuse the inoculum. Allow to gel.

After the agar has gelled, with sterile forceps place the antibiotic disks (usually three levels of concentration are available) one-half inch from the edge of the dish and at 12, 3, and 9 o'clock on the agar, as shown in Fig. 17-2-a. Test all of the antibiotics against all of the bacteria you have available. This will give you some interesting results for comparison of sensitivity. As a control, place in the center of each plate a filter paper disk (¼ in. or 6 mm) saturated in the sterile distilled water. Invert the plates and incubate at 37°C for 48 hours.

After 48 hours, examine the plates and look for a zone of inhibition. This is a clear area formed around the disk due to the inhibitory action of the antibiotic, as shown in Fig. 17-2-b.

The results you obtained with the disks are of a qualitative rather than a quantitative nature. There are many variables in this test such as the amount of the inoculum, the type of organism, the diffusion rate of the antibiotic, and the rate of deterioration of the antibiotic. Therefore, sensitivity is determined by the presence of a zone around any of the disks and not

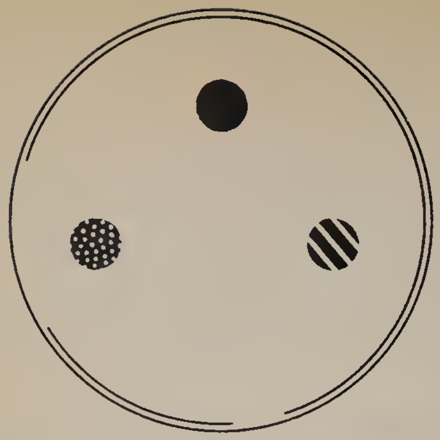


Fig. 17-2-a Technique for placing the disks on the agar

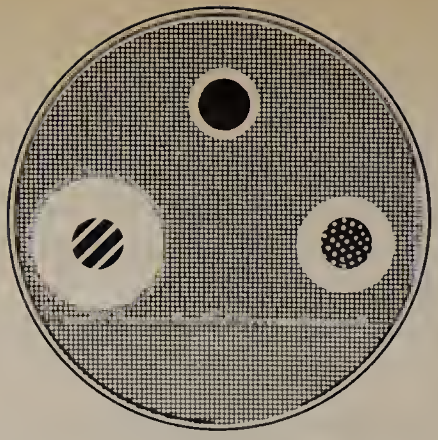


Fig. 17-2-b Zones of inhibition around the disks on agar plates

by the size of the zone. Consider the organism resistant if you find no zone on the plate. The organism is very sensitive to the antibiotic if there is a zone around the lowest level of concentration. An organism showing a zone around the disk of medium concentration, but not around that of the lowest, would be classified as sensitive or moderately sensitive. A zone around the disk of highest concentration indicates the organism is only slightly sensitive to the antibiotic. Now examine the plates and record the results as *resistant, slightly sensitive,* and *very sensitive* in the appropriate column in the table provided.

	Bacillus subtilis (Gram-positive)	*Escherichia coli (Gram-negative)*	*Proteus vulgaris (Gram-negative)*	*Serratia marcescens (Gram-negative)*
Penicillin				
Streptomycin				
Terramycin				
Chloromycetin				
Aureomycin				
Tetracycline				

If you use additional organisms or antibiotics, construct a table similar to the one provided and record your results.

You may find that some zones appear to have some growth in them. (a) Cite any reasons you can which would explain the presence of these bacteria in the zone. ....

....

Interpretation. Based on the results of your tests, rate the antibiotics you used in the order of their effectiveness against both Gram-positive and Gram-negative bacteria. ....

....

B Part 2 ANTIBACTERIAL ACTION OF AN ANTIBIOTIC AND A METAL. It has been demonstrated by research that certain pairs of chemically unrelated materials are often more active together than either compound would be alone. Antibiotics given in association with a metal, for example, may be more effective than when given alone.

Materials. Stock cultures of *Bacillus subtilis, Micrococcus epidermidis, Escherichia coli,* and *Proteus vulgaris;* solutions of penicillin, streptomycin, terramycin, and bacitracin (others may be substituted); forceps; 1-ml pipettes (one per sample of bacteria); bulb to fit pipette; sterile Petri dishes (one per metal and antibiotic and for water and antibiotic); incubator; ¼″ × 2″ filter paper strips; 500-ml Erlenmeyer flasks (one per bacterium tested); metallic salts may include zinc chloride, mercuric chloride, barium chloride, magnesium chloride, cupric chloride, iron (III) chloride; tryptose blood agar; nutrient broth; distilled water.

Procedure and observations. Prepare a broth culture of each bacterium by transferring a loopful of the stock culture to 250 ml of sterile nutrient broth in a 500-ml Erlenmeyer flask. Incubate for 24 hours at 37°C.

Identify sufficient sterile Petri dishes for testing the antibioics and metals against each bacterium. Identify a second set for testing the antibiotics and water against each bacterium. These will serve as controls. Using a pipette with a bulb (not your mouth) transfer 1 ml of each broth culture to the appropriate Petri dish. Pour 20 ml of sterile tryptose blood agar into each dish. Agitate the dishes to diffuse the inoculum. Allow to gel.

While the agar is gelling, saturate two strips of filter paper in each of the antibiotics to be tested. Use sterile forceps to hold the strips and allow any excess to drain off before placing them on the agar. Place the strips on the agar in the appropriate plate, as shown in Fig. 17-2-c. Then saturate a strip of filter paper in each metallic salt to be tested and place it over and at right angles to the antibiotic strip in the appropriate plate, as shown in Fig. 17-2-c. Saturate sufficient strips of filter paper in sterile water and place them over and at right angles to each antibiotic for controls.

Put all the plates in the incubator for 48 hours at 37°C. (Do not invert the plates.)

After 48 hours, examine the plates for zones of inhibition. This is a clear area formed around the strip due to the inhibitory action of the antibiotic and metallic salt. Show the results for the various antibiotics and metallic salts in the spaces provided on the next page. Label the strips as to type of antibiotic and metallic salt. (a) Explain why a control was used for each antibiotic. ....

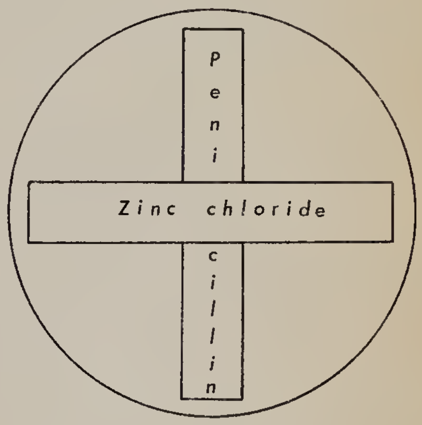


Fig. 17-2-c Technique for placing antibiotic and metallic salt strips on agar

....

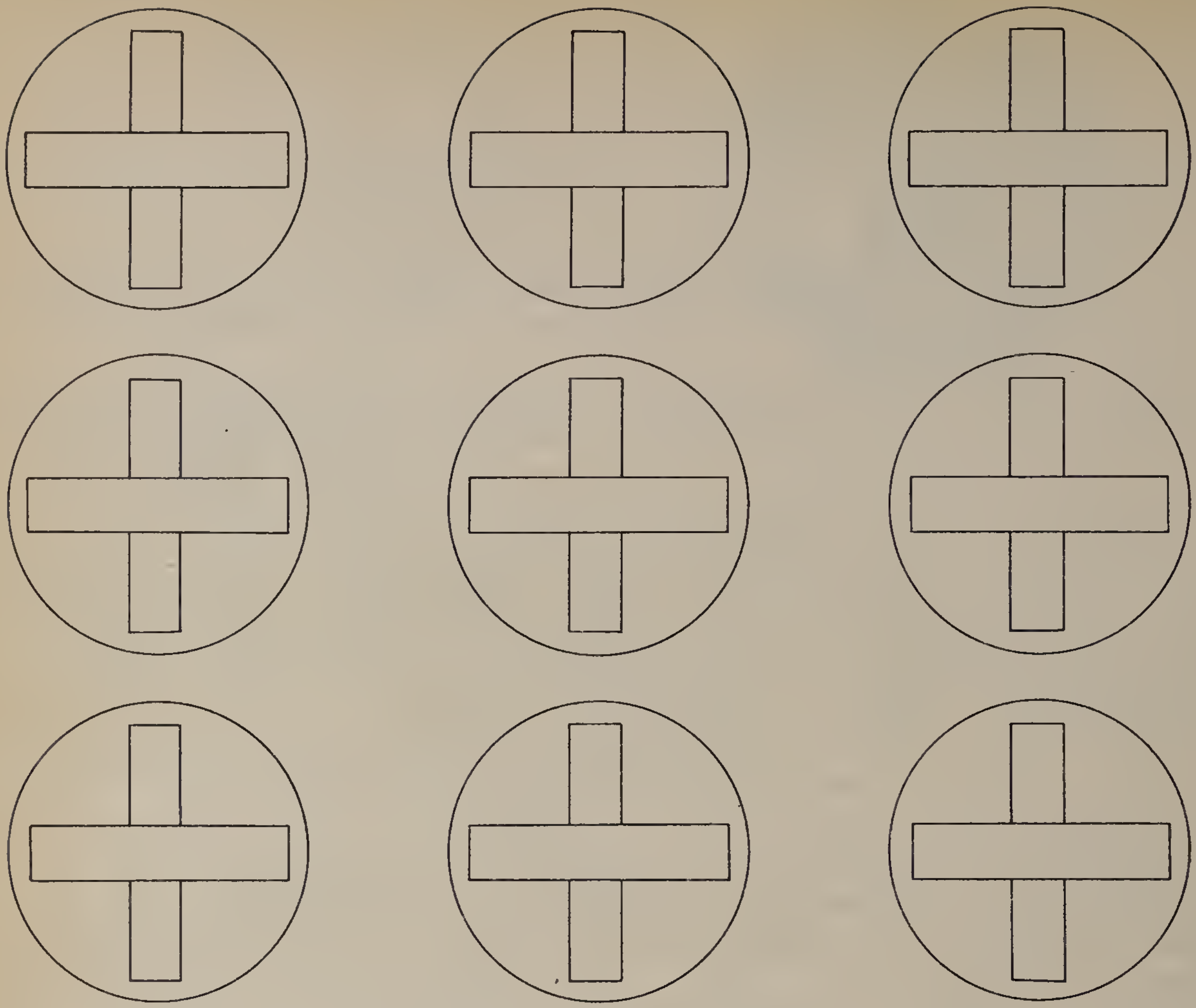
Interpretation. Cite evidence that demonstrates that some metallic salts increase the antibacterial effect of certain antibiotics, have no effect, or even inhibit the effect of the antibiotic.

....

....

B Part 3 SEARCHING FOR ANTIBIOTICS IN THE SOIL. Bacteriologists have found that many microorganisms in soil produce substances that inhibit bacteria and are necessary for their survival. Could these same substances have an antibacterial effect on disease-producing bacteria? Researchers are receiving and examining soil samples from all parts of the world in hope that they may find some antibiotics that can be used for the treatment of infections in animals as well as in human beings. A soil sample you collect may yield a mold that will produce an antibiotic.

Materials. Soil samples; stock cultures of *Escherichia coli, Bacillus subtilis, Proteus vulgaris,* and *Serratia marcescens;* sterile bottles for collecting soil samples; Petri dishes (five per sample,



Results of antibiotic and metallic salts against certain bacteria

one control); 1-ml pipettes (five per sample); 11-ml pipettes (four per sample); sterile water blanks (five per sample); balance; inoculating needle; 6-in. culture tubes; cotton for plugs; potato-dextrose agar; distilled water.

Procedure and observations. Collect soil samples from various areas such as your yard, a woods, a garden, or a field. Put the samples in your collecting bottles, number them, and tell where they were collected.

Put a piece of weighing paper on a balance, weigh it, and set the scale to weigh out 11 gm of soil sample. Weigh the soil sample and put it in a dilution bottle containing 99 ml of sterile distilled water. Recap and shake vigorously 50 times. You now have a 1:10 dilution of soil sample. With a sterile 1-ml pipette transfer this suspension to a Petri dish identified as *1:10.* Continue the dilution and plating of your sample as described in Investigation 16-5 until you have made dilutions through 1:100,000. Add 20 ml of potato-dextrose agar that has been previously liquefied and cooled to 45°C. Allow to gel, invert, and incubate at room temperature for 72 hours.

After 72 hours examine all the plates. Look for the two common soil molds, *Aspergillus* and *Penicillium.* These frequently produce substances that inhibit the growth of bacteria. Choose these in preference to other soil microorganisms. You can readily identify these by their characteristic low, velvety growth, and the green, black, brown, or yellow color. They do not produce distinct individual sporangia which you can see with the naked eye. Do not choose organisms with loose, cottony, white masses of mycelium, because they are not likely to produce antibiotics. Examine the organism under your microscope and use a key for identification.

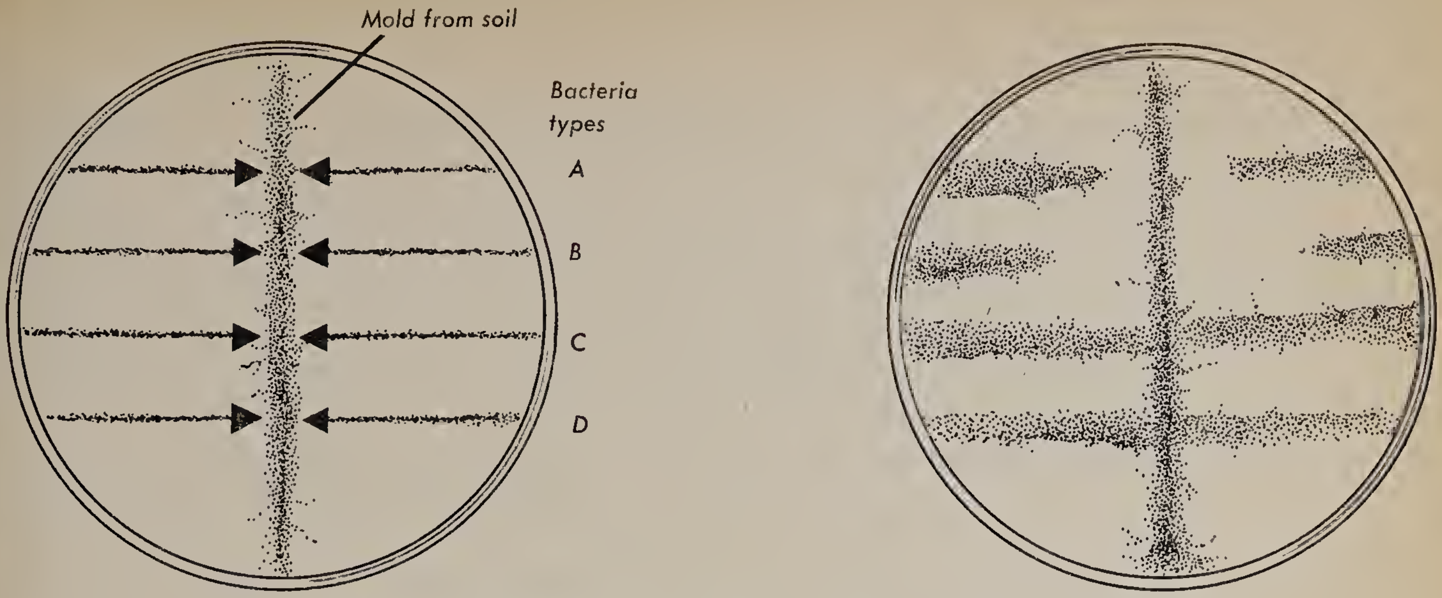


Fig. 17-2-d Action of an unknown antibiotic on test organism

After you have identified the molds you wish to use, discard the Petri dishes that are not to be used. With a sterile inoculating needle, transfer each of the molds to an individual sterile potato-dextrose agar slant. Allow these to grow at room temperature for 72 hours. After you are sure the molds you have cultured are pure cultures, you are ready to test them for antibiotic properties.

Pour 20 ml of the liquefied and cooled (45°C) potato-dextrose agar into Petri dishes (a sufficient number to test your molds for antibiotic properties), and allow to gel.

With a sterile inoculating needle, transfer a mold culture from a slant to a potato-dextrose agar plate by streaking the plate as shown in Fig. 17-2-d, left. Incubate the plates you streaked at room temperature for two days. Select several bacteria (four are used for each plate) from your stock cultures to be tested with the molds. With a sterile inoculating needle transfer the bacteria to the plate by streaking at right angles to the central streak. First streak the bacteria across the surface of the agar from the left side of the dish to the original mold streak. Sterilize the needle and repeat the process with another sample of the *same* bacteria, streaking from the right side to the original mold streak, as shown in Fig. 17-2-d, left. Follow this procedure for all plates you prepare. Incubate the plates at room temperature for 72 hours.

After 72 hours examine your plates. Look for any clear areas such as those shown in Fig. 17-2-d, right. These indicate that you have isolated a mold that has an antibiotic effect on the test organisms.

Interpretation. Discuss the results of the antibiotics you tested and their effect, if any, on the test organisms. ....

....

....

....

Unicellular Organisms—The Protozoans 18-1

Purpose To study specialization in an organism at the cellular level and to observe the activities of three groups of protozoans; to study diversity among protozoans and to learn to culture them.

The protozoans include a variety of organisms usually listed in the protist kingdom. Some of these unicells are capable of making their own food. Others have developed methods of taking food through their membranes or special organelles designed for this purpose. Each of the organisms you will study has developed a special means of locomotion.

A Part 1 THE AMEBA. The ameba is an interesting single-celled protist capable of maintaining life with a minimum of organelles.

Materials. Culture of ameba; microscope; slides; cover glass.

Procedure and observations. Put a drop of the sediment from the ameba culture on a slide and put a cover glass over it. Examine under low power, observing the movement of the organism. You may have to cut down the light entering the microscope to see the ameba clearly. Make three drawings of the locomotion in the ameba at 30-second intervals in the space provided. Use arrows to indicate the flow of cytoplasm in the cell. Watch closely the streaming of the cytoplasm. (a) Where is the most rapid flow? .... (b) Explain how the streaming illustrates phase reversal in a colloid. ....
(c) Does the cell form more than one pseudopodium in one direction? .... Study the cell carefully to determine whether the ameba engulfs food. (d) If so, describe what happens. .... Watch for a clear bubble in the cell. (e) Describe its action. ....
This is the contractile vacuole, which eliminates the liquid wastes that build up in the cell. Locate the nucleus. (f) Is it stationary or does it change position? ....
(g) Account for your answer. ....

Ameboid movement (L.P.)

Make a drawing of the ameba in the space provided and label: **plasma membrane, pseudopodium, endoplasm, ectoplasm, food vacuole, contractile vacuole, nucleus.**

Structure of the ameba (L.P.)

A Part 2 THE EUGLENA. The euglena serves as an excellent example of why many biologists recognize a protist kingdom and include in it those ancient organisms that are neither distinctly plant nor distinctly animal.

Materials. Culture of euglena; microscope; slides; cover glasses; colored pencils.

Procedure and observations. Put a drop of euglena culture on a slide. Put the cover glass in place and examine under the low power and high power of your microscope. Study the manner in which the cell moves by extension and contraction. This is called *euglenoid movement.* Make three drawings of euglenoid movement, showing consecutive contractions, in the space provided. Use arrows to indicate the direction of movement.

(a) In what direction does the cell move? .... Study the cell as it moves and try to locate the flagellum extending from the cell. (b) How does it enable euglena to move? ....

Examine the cell under high power and observe the organelles. (c) What are the green structures? .... (d) Describe the eyespot. .... (e) Why is the eyespot of value to an organism like the euglena? .... Focus your microscope with the fine adjustment and try to locate the diagonal cytoplasmic strands in the cell. The gullet and reservoir are located at the

Euglenoid movement (H.P.)

anterior end of the cell. (f) Of what value is the gullet to the euglena? ....

....

Make a drawing of the euglena in the space provided and label: **anterior end, posterior end, flagellum, gullet, reservoir, eyespot, chloroplast, nucleus.**

Structure of euglena (H.P.)

A Part 3 THE PARAMECIUM. The paramecium, or "slipper animalcule," is often found in the scum that forms on the surface of stagnant water. When you examine it under the microscope, you will note its rapid movement. Actually, it moves very slowly, but the magnification of the microscope appears to increase its speed.

Materials. Culture of paramecium; microscope; slides; cover glasses; acetic acid; methyl cellulose.

Procedure and observations. Put a drop of the scum from the paramecium culture on a slide and put a cover glass over it. Examine under low power and observe the organisms as they move about on the slide. Make three drawings of the paramecium in the space provided, showing the spiral path of the organism as it darts about on the slide. (a) Examine the cell carefully and determine why the cell moves in this manner. ....

....

Movement of paramecia (L.P.)

In order to quiet the animals for further study, you may prepare traps of cotton fibers or use a drop of methyl cellulose on the slide. Now examine the paramecium under high power. (b) Is the cell flexible? .... (c) Can it change its shape like the ameba? .... (d) If not, explain why. ....
.... Examine the edge of the cell and locate the cilia. (e) What is their function? ....
Locate the oral groove and note that cilia also line it. (f) What is the function of these cilia?
.... Look for any food vacuoles. (g) In what direction do they move? .... (h) Explain your answer. .... Look for the clear circles and numerous canals leading to them at either end of the cell. Watch their action for a few minutes. (i) Describe how they work. ....

....

Study the area of the cell just posterior to the gullet. Watch for the opening of the anal pore and discharge of material. The trichocysts lie just inside of the pellicle. Add a drop of acetic acid at the edge of the cover glass and observe what happens to the trichocysts. (j) Record your observations. ....

....

Many cells contain several micronuclei. (k) Count and record the number of micronuclei in your specimen. ....

Make a drawing of the paramecium in the space provided and label all the organelles you can locate.

Structure of paramecium (H.P.)

B Part 4 DIVERSITY AMONG THE PROTOZOANS. You will find protozoans wherever moisture is available. They occupy rivers, ponds (fresh or stagnant), lakes, and almost any stream. Many live in mud, marine waters, brine pools, hot springs, and snowdrifts. Many are unicellular; others are colonial.

Materials. Collecting jars; microscope; slides; cover glasses; pipette; metric ruler.

Procedure and observations. Collect protozoans from puddles, roadside ditches, and any place where water collects temporarily, as well as from permanent bodies of water. Put each sample in a separate jar and assign a collection number. Record the number, place collected, depth of water, and condition of water in your field notebook.

When collecting surface water from a pond, put some of the plants, usually filamentous algae or duckweed, in the jar. Then fill it with the water. Some of the jars may contain a small amount of the vegetation: others may be half full. Be sure to collect some of the debris from the bottom of the pond. This usually includes dead plants, twigs, and leaves. Put some of the sediment in some of the jars; then fill them with the water. Do not hesitate to collect protozoans in the winter. They often remain on the debris in the bottom of the pond. If the pond is frozen, chip a hole and collect some of the debris. Be sure to fill your collecting jar with some of the water.

After you return to the laboratory, remove the lids and place the jars on a shelf or windowsill that receives a moderate amount of light. Intense light heats the jars too much and will kill most of the bacteria, which serve as food for the protozoans. Allow the cultures to stand for 24 hours so that the protozoans can adjust to the new environment.

At the end of the 24-hour period transfer a drop of the surface culture of each jar to a microscope slide and mount with a cover glass. Examine the drop with low power or high power of the microscope. Some specimens are large enough for study under low power. Others may be smaller and will have to be studied under high power for accurate identification. Compare the specimens with those in Fig. 18-1-a. If you do not find an illustration of your specimen, look in various books on protozoans or use an identification key. Since some organisms will be classified as algae, check your specimens with Fig. 18-1-a.

Protozoans are identified by their method of locomotion, size and shape of cells, and distinctive organelles. Study each of your specimens and record all data relating to them on the chart.

Specimen number	*Place collected*	*Water condition*	*Method of locomotion*	*Shape of cell*	*Distinctive organelles*	*Class*	*Name of organism*

Examine a drop of the culture from various depths and at the bottom of each jar. (a) Do you find some of the same organisms at all depths? .... (b) Is there a great variation in the number of organisms at each depth? .... Be sure you examine cultures from the sides of the jars. (c) What type of protozoans are most common in these cultures? ....

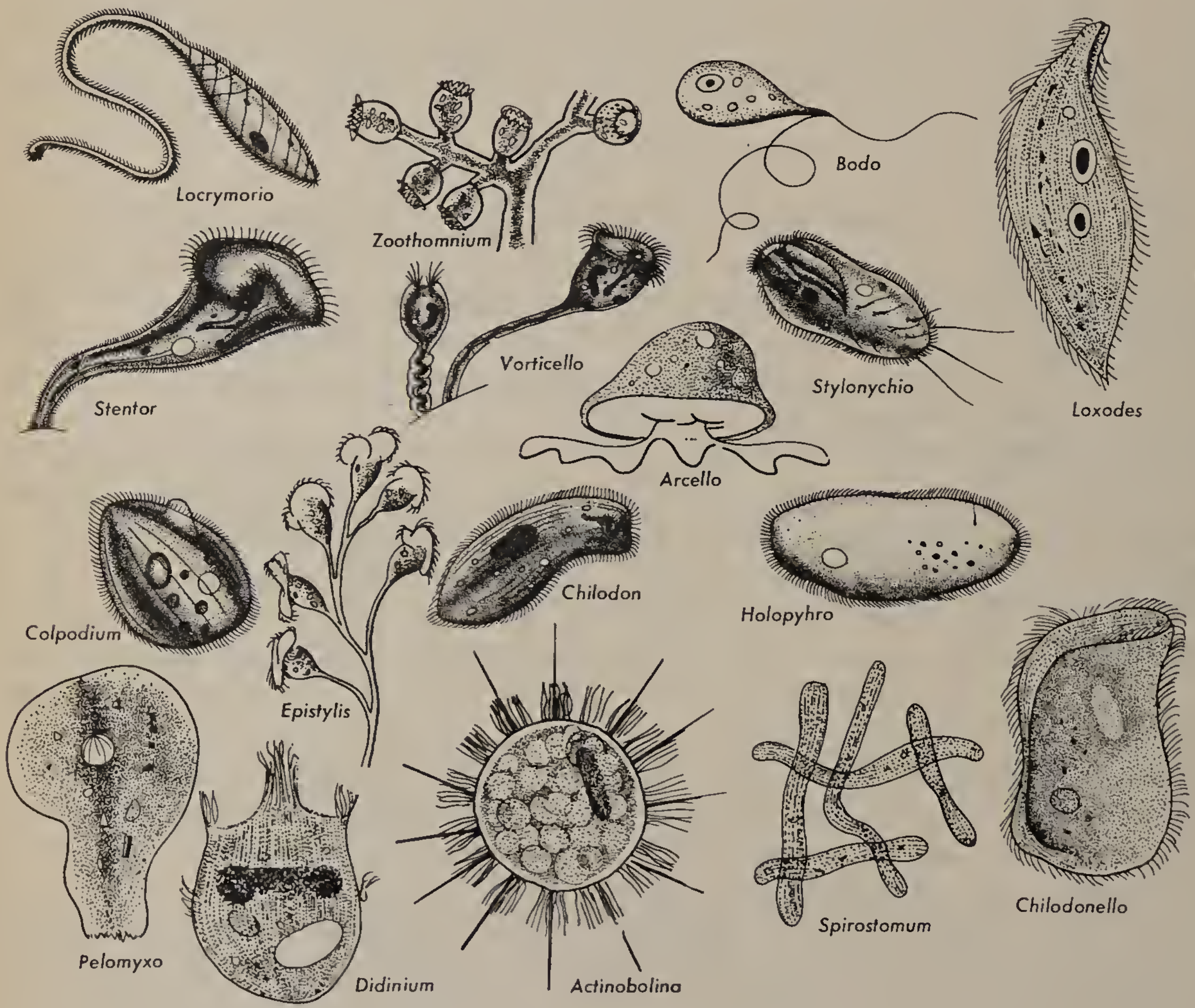

Locrymorio
Zoothomnium
Bodo
Loxodes
Stentor
Vorticello
Stylonychio
Arcello
Colpodium
Epistylis
Chilodon
Holopyhro
Chilodonello
Pelomyxo
Didinium
Actinobolina
Spirostomum

Fig. 18-1-a Miscellaneous protozoans

Since the protozoan population in a culture may change from time to time, continue to examine your cultures over a period of three weeks. (d) List the changes you observe in the populations of the various cultures. ....

....

.... (e) Account for these changes. ....

....

Interpretation. In your examination of various cultures, you undoubtedly found many different protozoans representing high degrees of cell specialization. In what way are these adaptations and cell specializations important in maintaining a community of organisms in a microscopic protozoan society (microcosm)? ....

....

....

....

B Part 5 CULTURING PROTOZOANS. In this Part you will learn how to grow protozoans in the laboratory so that they are available in large numbers for further study over a long period of time.

Materials. Cultures of ameba, paramecium, and euglena; 7 culture dishes; battery jar; wheat kernels; timothy hay; pipette or micropipette; distilled water.

Procedure and observations. Section 1 *Culturing ameba.* Culture dishes are best for amebas because of the large amount of surface for the depth of the water. Fill the dish with distilled water and add three to five wheat kernels and ten pieces of timothy hay, about one inch long, which have been boiled in water five minutes. Cover the culture and set it aside for about two weeks. If stacking finger bowls are used, several cultures can be prepared and one set on the other, with a cover on the top dish. During the two-week period, bacteria will multiply and provide a source of food for the amebas. If a culture of amebas is available (one used for the laboratory study of the ameba), transfer a few animals to each of the new cultures with a clean pipette. If you wish to collect amebas from a pond, bring in several bottles of pond water containing decaying water plants. Transfer this to culture dishes and, after about one week, examine the sediment under the microscope each day. If amebas are present, they should appear in considerable numbers within two weeks. An ameba may then be transferred to the new cultures with a clean pipette. Keep the cultures at room temperature and in reduced light.

Section 2 *Culturing paramecia.* Either stacked culture dishes or battery jars are excellent for culturing paramecia. If a culture dish is used, fill it with distilled water, then add six kernels of wheat or rice and 15 pieces of timothy hay, about one inch long, which have been boiled for five minutes. Cover the dish (several may be stacked) and set it aside for five days to one week. The paramecia may then be added. These may be taken from a stock culture, purchased from a supply house, or taken from cultures obtained from a pond. If freshly collected paramecia are used, they should be transferred with a clean pipette, using care not to introduce other protozoans which might contaminate the culture. A micropipette is best, since a single cell can be picked up. You can prepare one of these by heating a piece of glass tubing in a burner and pulling out the tube from both ends while the glass is hot. After the tube cools, break the narrow, pulled section to produce a small-bore, tapering pipette of the desired length. Fasten a pipette bulb on the large end. Paramecia should multiply rapidly in the culture for about two weeks. It is then necessary to add more food, or better still, to transfer animals to a new culture. Maintain paramecium cultures at room temperature or in a warm place (80-85°F) and in a north window where they do not receive direct sunlight.

Section 3 *Culturing euglena.* Pour a concentrated culture of euglena into a culture dish. Add distilled water or aged tap water to fill the dish. Cover and place in a well-lighted area. Fill a six-inch battery jar with distilled water and add about 100 kernels of wheat or rice, which have been boiled for five minutes. Set the culture in a window for about one week, then add euglenas from the culture dish. Or you may collect euglenas in a pond and transfer them to the culture. In collecting euglenas, look for small pools of greenish water. Examine the water with a microscope and, if euglenas are present (other organisms give water this color), transfer some to the culture with a clean pipette or micropipette. The culture should last at least one month. Add food every two weeks as long as the culture is maintained. When the euglenas begin to die out, transfer some to a new culture.

Interpretation. Discuss the advisability of establishing new cultures from existing cultures if protozoans are to be grown over a period of time. ....

....

....

Purpose To study the responses of protozoans to various stimuli.

Although microorganisms lack the specialized nervous tissue found in higher forms of life, their protoplasm is sensitive to various environmental stimuli.

B Part 1 RESPONSES TO STIMULI. In this Part you will determine whether various protozoans respond positively or negatively to chemicals, electricity, gravity, heat, touch, and light.

Materials. Culture of ameba, of paramecium, of *Vorticella* or *Stentor;* microscope; slides; cover glasses; U-tube; 2 dry cells; 4 test tubes; stoppers for tubes; dark paper to cover tubes; matches; spun glass; piece of cardboard; salt crystals; vinegar; ink; carmine red solution.

Procedure and observations. Section 1 *Response to chemicals.* Add a drop of paramecium or ameba culture to a slide and focus under low power. Now add a few salt crystals to the culture and observe a cell closely. (a) How does it react? ....

(b) What occurs within the cell? .... (c) Explain why this reaction occurs. ....

Clean the slide and add another drop of ameba or paramecium culture. Add a drop of vinegar. Using low power, observe the activity of the organism. (d) What response do the cells make to the acid? .... Bacteria serve as food for paramecia. (e) In view of the response you have just observed, would you expect that bacteria live in an acid or an alkaline medium? ....

Prepare a slide of paramecium in debris containing food. Observe the behavior of the cells under low power. (f) What do you find? ....

Prepare a slide of the paramecium culture and put a cover glass in place. Now put a drop of ink under the edge of the cover glass. Observe under low power the reaction that occurs in the paramecium as the ink diffuses through the water. (g) What happens within the cell?

....

Prepare another slide of paramecium culture and place a drop of carmine red solution at the edge of the cover glass. As the particles of stain approach a paramecium, notice the action of the cilia along the oral groove. Study the cell structures carefully. (h) What happens to the solution? ....

Section 2 *Response to electricity.* Fill a U-tube with a concentrated culture of paramecium. Insert the wires from two dry cells, which have been connected in series, into opposite ends of the U-tube containing your culture. This apparatus is shown in Fig. 18-2-a. Observe the activity of the paramecium. (i) Where are the cells concentrated in the U-tube? ....

....

Now reverse the wires and observe what happens. (j) Do the paramecia migrate to the negative pole? .... (k) What would this indicate to you about the charge on the surface of the paramecium? ....

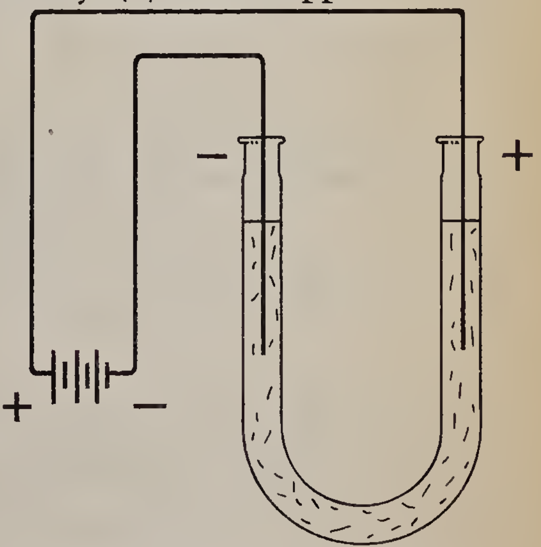


Fig. 18-2-a Apparatus for testing paramecium response to electricity

Section 3 *Response to gravity.* Pour a culture of paramecium into four stoppered test tubes. In order to demonstrate that the response is due to gravity rather than light, cover two of the tubes with dark paper. Put a covered and uncovered tube in a vertical position. Put the remaining two cultures in a horizontal position. Allow the cultures to remain in their respective positions for 10 minutes. Examine each of the tubes. (l) Where are the paramecia concentrated in the uncovered tube in the vertical position? ....

.... (m) in the covered tube? ....

(n) in the uncovered horizontal tube? .... (o) the covered horizontal tube? .... (p) Does light have any effect on the response to gravity? ....

Section 4 *Response to heat.* Prepare a slide of paramecia and check under low power to be sure that several animals are present. Remove the slide and heat one side of it gently with a match for a few seconds. Examine the slide under low power at once. (q) How do the paramecia react to the heat? ....

Section 5 *Response to touch.* Put a drop of *Vorticella* or *Stentor* culture on a slide for microscopic study. Locate one of the organisms in the field. (r) Now gently tap the slide and observe and record the reaction of your specimen. ....

.... Place a drop containing paramecia on a slide and observe their reaction as they come in contact with one another. (s) Record your observations.

.... Put a few strands of spun glass just under the cover glass of an ameba culture. (t) Observe and record the reaction of the ameba. ....

Section 6 *Response to light.* Cover half of the culture bowl with a piece of cardboard. Illuminate the other half of the bowl. Allow the culture to stand for 10 minutes. Remove the cardboard and illumination and examine the culture immediately. (u) Where are the euglenas concentrated? .... (v) What structure is stimulated by the light?

.... (w) How does the euglena use the light? ....

.... You may also study the reaction of the euglena to light under the microscope. Prepare a slide of euglena culture and examine under low power. Now close the diaphragm to its smallest opening. (x) Observe and record the activity of the organisms. ....

....

Put a drop of ameba culture on a slide and locate one organism in the field. Darken one side of the field with a piece of cardboard. Observe the changes in the direction of ameboid movement. (y) Record your observations. ....

....

Interpretation. Summarize the responses of unicellular organisms to various stimuli in the environment. Discuss the possible survival value of each response. ....

....

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Structure and Distribution of Common Molds 19-1

Purpose To study the structure, nutrition, and reproduction of bread mold and to examine other molds.

Bread mold, a black mold, is one of the most common and widely distributed of all the fungi. You have probably seen it as a cottony growth on bread and other foodstuffs. *Aspergillus* and *Penicillium,* two blue-green molds, are also common and widely distributed. *Neurospora* is a salmon-pink mold often found on bread. It has gained a great deal of prominence because of its use in research in the field of genetics.

A Part 1 GROWING BREAD MOLD. Under ideal conditions of moisture, darkness, food supply, and the right temperature, you can grow your own culture of bread mold for study.

Materials. Culture of bread mold; microscope; dissecting needles; slides; cover glasses; forceps.

Procedure and observations. To prepare a bread-mold culture, trim a piece of bread to fit into a covered dish (a moist chamber or culture dish is good). Flood the bread with water, then drain off the excess water so none is left standing in the dish. Leave the dish open for about an hour, then cover it and place it in a dark, cool place for a week or more. (If a stock culture of bread mold is available, dust some spores onto the bread and cover the dish immediately.) When the mold has developed, search for an individual mold plant, which you can recognize as a cluster of tiny grayish threads with black knobs at the ends. Remove a plant from the culture and lay it in a drop of water on a slide. Add a cover glass and examine with low power. Without magnification, bread mold looks like a tangled mass of hyphae. (a) What is the entire mass of hyphae called? ....

Fill in the table below after you have identified the three kinds of hyphae revealed by magnification.

Kind of hyphae	*Function*

(b) How do the mold plants obtain food? ....

Some mold plants have probably grown along the sides of the dish in your culture, without contact with the food supply. (c) How are these plants nourished? ....

.... (d) Do you detect an odor in your culture? ....

.... (e) Explain your answer. ....

....

Examine a sporangium at the tip of an ascending hypha with high power. Find the thin sporangium wall, which may have burst open and released spores. Examine the spores and notice the tremendous number produced. (f) What advantage is offered by the fact that the sporangia develop on top of the ascending hyphae? ....

....

Draw the bread mold in the space provided. Label: **rhizoids** (*descending hyphae*), **stolon** (*transverse hypha*), and **sporangium.** Draw a sporangium as seen under high power in the space provided. Label: **sporangium wall, spores, ascending hypha.**

Bread mold (L.P.)

Sporangium (H.P.)

A Part 2 **BLUE AND GREEN MOLDS.** The drug penicillin is extracted from one species of *Penicillium. Penicillium* is found as a green growth upon almost any form of decaying organic matter. *Aspergillus* is a common blue-green mold found on bread, cheese, preserved and decaying fruit. It is green in color in the immature stage, but becomes reddish yellow or reddish brown in the mature stage. In this Part you will examine these two molds and become acquainted with their structure.

Materials. Stock culture of *Penicillium notatum* and *Aspergillus niger;* Petri dishes (100 × 20 mm); microscope; slides; cover glasses; inoculating loop; hand lens; Bunsen burner; autoclave or pressure cooker; hot-air oven; Sabouraud's dextrose agar.

Procedure and observations. Prepare the Saubouraud's dextrose agar according to the directions on the bottle, then sterilize in the pressure cooker or autoclave for 15 minutes at 15 pounds pressure. Pour 30 ml of the sterile agar into sterile Petri dishes and allow to gel.

Using a sterile inoculating loop, transfer a loop of the *Penicillium notatum* culture to the center of the sterile gelled agar. Rotate the loop on the surface of the agar to remove the mold culture. Then, in the same manner, transfer a loop of the *Aspergillus niger* culture to a second Petri dish. Place the inoculated plates in a dark, cool place for a week or more. At the end of this time, use a hand lens to examine the colonies.

(a) Describe the colony formed by *Penicillium notatum.* ....

.... (b) *Aspergillus niger.* ....

....

Remove the lid from the *Penicillium* culture dish and place the culture on the microscope stage for examination. Focus on the edge of the colony with low power and locate the fruiting bodies (*conidia*) which are produced in chains at the tips of certain hyphae called *conidiophores.* (c) Describe the hyphae. ....

(d) How do they differ from the hyphae produced by bread mold? ....

.... (e) Describe the arrangement of the conidia. ....

In the same manner, examine the culture of *Aspergillus.* (f) Describe the hyphae. ....

.... (g) Are they the same in structure as *Penicillium* hyphae?

.... (h) Explain your answer. ....

.... (i) Describe the conidia. ....

(j) Are they similar to those produced by *Penicillium?* .... (k) Explain your answer. ....

B Part 3 CULTURING NEUROSPORA. *Neurospora* grows as a spreading mycelium over the host material. The spores germinate rapidly and you can observe the growth of the hypha 24 hours after inoculation of the medium.

Materials. Culture of *Neurospora;* Difco's Bacto-*Neurospora* Agar; microscope; Bunsen burner; pressure cooker or autoclave; hot-air oven; Petri dishes; inoculating needle; slides; cover glasses; metric ruler.

Procedure and observations. Prepare the Bacto-*Neurospora* Agar as directed and sterilize the medium in the pressure cooker or autoclave for 15 minutes at 15 pounds. Pour a thin layer of the medium into sterile Petri dishes and allow to gel. With a sterile inoculating needle, transfer a few spores of *Neurospora* to the center of the gelled agar medium. Incubate the inoculated Petri dishes for 24 hours at room temperature. After 24 hours examine the inoculated plates.

(a) Record your observations. ....
Make a drawing of the plate in the space provided.

Inoculated plate after twenty-four hour incubation

Neurospora (H.P.)

Remove the lid of the Petri dish and place a cover glass on the plate so that half of it covers the tip of a hypha and half is over bare agar. Gently press the cover glass with your fingertips or a pencil eraser to remove any air and to permit moisture in the agar to move up under the cover glass. Put the Petri dish on the microscope stage and observe the hyphal tip under high power. (b) Record your observations. ....
Move your Petri dish and examine the rest of the mold. (c) What type of growth does *Neurospora* have? .... (d) Do you find evidence of walls separating the cells? .... (e) Do you think these are true walls? ....

(f) Explain your answer. ....

(g) Does cytoplasm flow more rapidly in one area than in another? .... (h) Account for your answer. ....

.... (i) What structures can you locate in the hyphae?

.... Draw *Neurospora* in the space provided.

Measure the cells at the tip of the hyphae and the older cells at various intervals. Compare the length of these cells. (j) Which cells grow in length? ....

Interpretation. On the basis of your examination of *Neurospora* during its growth, describe how it very quickly forms a growth over the agar. ....

....

B Part 4 COLLECTION AND IDENTIFICATION OF DIFFERENT SPECIES OF MOLD. Certain molds are able to survive under acid conditions and in high concentrations of sugar or salt. They grow as parasites and as saprophytes and cause considerable damage to food products. Some of the molds are used commercially to produce cheese. In this Part you will become acquainted with various molds found on your food.

Materials. Fruits and vegetables with mold growths; microscope; forceps; slides; cover glasses; identification keys to the molds.

Procedure and observations. A visit to your grocer will produce several fine specimens of mold, since oranges, grapefruit, peaches, and other fruits and vegetables often develop molds in shipping. Roquefort and Camembert cheeses depend on mold growth for their fine flavor. Examine an area containing dark lines, and you will find the mold. Obtain the discarded parts of cheese from your grocer and you will find other molds.

With your forceps, take a small piece of the mold you are going to study and put it in a drop of water on a slide. Put a cover glass over it. Examine all its parts under low power. Make a drawing on separate paper of the mold plant. Identify each one as to genus and write the name below your drawing. Summarize your observation of each mold you examine in the table below.

Name of mold	*Place collected*	*Color of mycelium*	*Color of sporangia*	*Other characteristics*
1.				
2.				
3.				
4.				
5.				

Interpretation. From your experience in collecting and identifying the molds, discuss the frequency of contamination, the types of fruits and vegetables contaminated, and the occurrence of the various kinds of molds. ....

....

....

....

Study of Yeast

19-2

Purpose To study the structure, nutrition, and reproduction of yeast and to study fermentation of various sugars and starches.

The yeasts are among the most familiar and economically important of the fungi. When you use a package of dried yeast in baking, millions of yeast cells are released into the dough. During fermentation, they release carbon dioxide and alcohol. The bubbles of carbon dioxide cause the dough to rise, and the alcohol is driven off during baking. Yeast is also used to ferment various carbohydrate mashes in the manufacture of commercial mashes.

A Part 1 STUDY OF YEAST. Yeasts are unlike other fungi in that the plant body, instead of having hyphae, consists of a single spherical or oval cell enclosed by a thin cell wall. The wall is absent in very young cells. The cell wall begins to appear as a delicate membrane in cells that are one-third grown. Vacuoles are inconspicuous in young cells, but they occupy the greater portion of mature cells and are the most conspicuous structure in the cell. Cells mature a few hours after they are produced. The cytoplasm contains numerous granules of reserve food. The nucleus is very small, and it is difficult to locate unless the cell is stained with methylene blue or iodine stain.

Yeast cells form buds by producing a bulge on the side of the cell. The bulge grows rapidly and may produce other buds while attached to the mother cell. Thus, you may find chains of cells of various sizes in your culture.

Materials. Package of yeast; microscope; slides; cover glasses; fermentation tubes or 250-ml flasks; cotton for plugs; dropping pipettes; stirring rod; 10% molasses solution; methylene blue or iodine stain.

Procedure and observations. Prepare a yeast culture as follows: Make 500-ml of 10% molasses solution (10 ml of molasses to 90 ml of water). Mix the syrup with the water, then add half a package of yeast and stir it. Divide the culture in several flasks or bottles, filling each about half full. Plug the flasks with cotton. If fermentation tubes are available, prepare several as shown in Fig. 19-2-a (left).

Using a pipette, put a drop of the yeast culture on a microscope slide, add a small drop of methylene blue or iodine stain, cover with a cover glass and examine under high power. Make drawings to show the general appearance of several yeast cells in the space provided. Make an estimate of the number of yeast cells in the field under high power. (a) How many cells did you estimate? ....

Set the flasks or tubes in a warm place overnight. Examine the cultures the next day. (b) What evidence do you find that fermentation is occurring? ....

Remove the plug from the flask and smell the culture. (c) What odor do you detect? ....

.... Taste a drop of the liquid. (d) How does it taste?

.... (e) Explain your answer. ....

.... (f) What substances are produced during fermentation?

....

With a pipette, put a drop of the liquid on a microscope slide and add a small drop of methylene blue or iodine stain. Add a cover glass and examine under high power. (g) Estimate the number of cells in the high-power field. ....

Yeast cells first day (H.P.) | Yeast cells second day (H.P.) | Yeast cell and buds (H.P.)

(h) How does the number compare with the one in the fresh culture? ....

....

(i) Account for any difference. ....

Draw several cells to show their general appearance in the space provided. (j) How have the cells changed during fermentation? ....

Look for cells that have buds on them. Draw these cells in the space provided. Label: **bud, vacuole, cell wall, nucleus.**

B Part 2 FERMENTATION BY YEASTS. Yeasts ferment materials more rapidly if the concentration of sugar is high and that of oxygen is low. The energy obtained during fermentation under anaerobic conditions is sufficient to maintain cellular activities. The extent of fermentation depends on the species of yeast in the particular medium. Commercially *Saccharomyces cerevisiae* is used in baking and *Saccharomyces ellipsoideus* is used in wine making. In this Part you will determine the extent to which these species of yeast plants can ferment various sugars and starches.

Materials. Yeast suspensions of *Saccharomyces cerevisiae* and *Saccharomyces ellipsoideus;* 2 dropping pipettes; 12 fermentation tubes; six 250-ml flasks for the sugar and starch solutions; cotton for plugs; 100 ml of each of the following: maltose (10% solution); sucrose (10% solution); lactose (10% solution); glucose (dextrose) (10% solution); potato starch (10% solution); corn starch (10% solution); brom thymol blue solution.

Procedure and observations. Prepare two series of fermentation tubes for the sugar and starch solutions. Number one series *1A-6A* and identify each tube with the carbohydrate solution it is to receive. Number the second series *1B-6B* and identify each tube with the solution it is to receive. Fill each of the properly identified fermentation tubes with the following solutions: maltose, sucrose, lactose, glucose (dextrose), potato starch, and corn starch. Be sure that they are filled with the solution as shown in Fig. 19-2-a, left.

Using a clean pipette, transfer 1-2 ml of the *Saccharomyces cerevisiae* suspension to each of the fermentation tubes in series 1A-6A. Using another clean pipette, transfer 1-2 ml of the *Saccharomyces ellipsoideus* suspension to each of

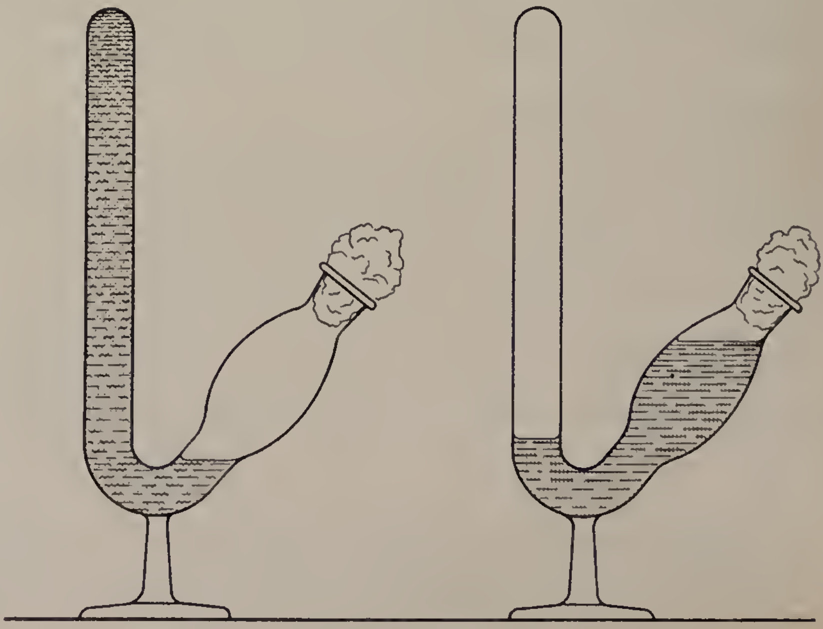


Fig. 19-2-a Yeast fermentation tubes

the fermentation tubes in series 1B-6B. Plug the tubes with cotton and place the cultures in a warm place overnight. Examine them the next day for evidence of fermentation and record the results in the table provided, use a plus (+) if fermentation occurred and a minus (−) if no fermentation occurred.

Food Supplies	*Fermentation*	
	Saccharomyces cerevisiae	*Saccharomyces ellipsoideus*
Maltose		
Sucrose		
Lactose		
Glucose		
Potato starch		
Corn starch		

(a) What evidence did you find in the fermentation tubes that fermentation had occurred?

Add a few drops of brom thymol blue to the mixture in which gas was produced and observe any change that occurs. (b) What change, if any, occurred?

(c) What does this indicate?

(d) From what substance in the solution was it produced?

(e) Account for your answer to *e*.

Smell the fermented solutions. (f) What do you detect?

Now taste the solutions you determined were fermented. (g) How did they taste?

(h) Account for your answer.

Measure the amount of gas in milliliters produced in each of the tubes in which fermentation occurred and prepare a bar graph on graph paper showing the amount collected in the tube.

Interpretation. On the basis of your observations, explain how the two species of yeast differ in their ability to ferment various sugars and starches.

The Mushroom

Purpose To become familiar with the structure of a mushroom and to study its spore-bearing structures.

Mushrooms, like all fungi, lack chlorophyll. They are dependent on organic material in the soil for their food supply. Edible mushrooms are considered delicacies, but many mushrooms are deadly poisonous.

A Part 1 STRUCTURE OF A MUSHROOM. We usually think of a mushroom in terms of its umbrella-shaped reproductive structure, which appears suddenly, usually after a rain. The vegetative body is the filamentous *mycelium* in the ground, composed of many branching *hyphae.* Hyphae mass together to form the *stipe, cap,* and *gills* of the familiar mushroom. *Spores* are discharged from the gill surfaces. The veil-like *annulus* below the cap was attached to the rim of the cap and covered the gills before the mushroom opened.

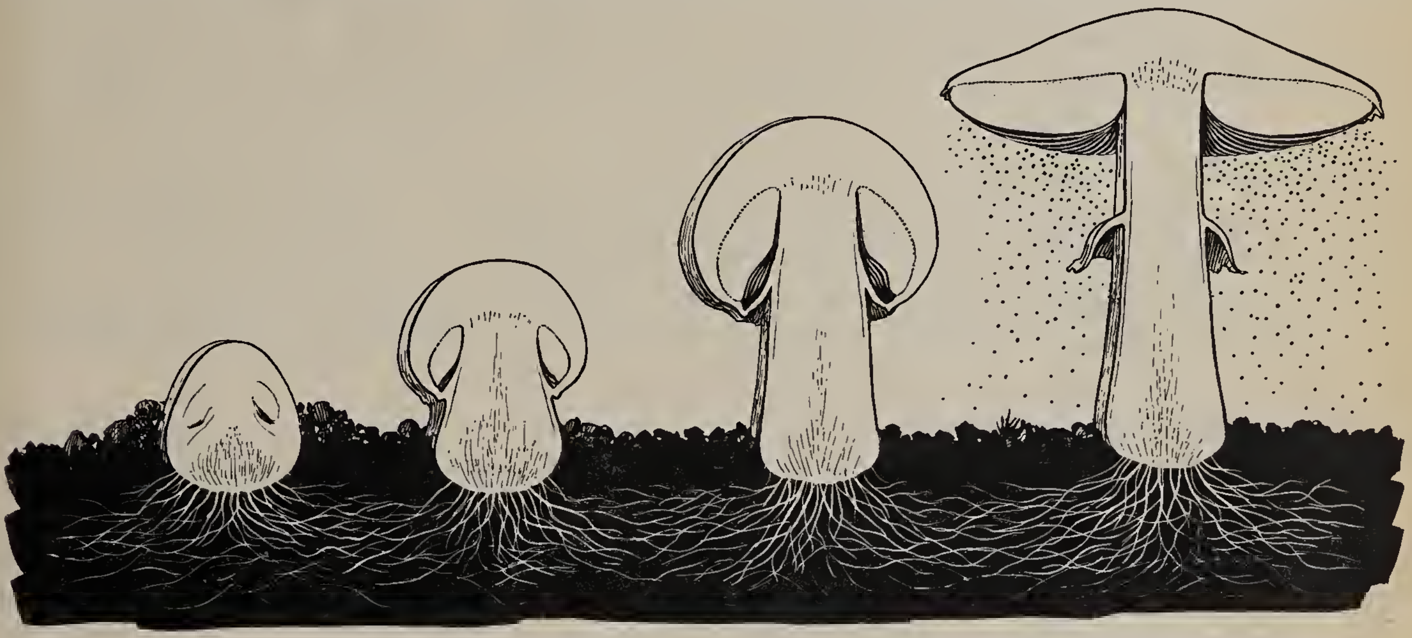


Fig. 19-3-a Field mushroom (*Agaricus campestris*)

Materials. Fresh mushrooms; scalpel; hand lens.

Procedure and observations. Figure 19-3-a shows the various parts of a mushroom, including those below the ground. Label the **hyphae, stipe, annulus, cap, gills, spores.**

Now section a mushroom lengthwise through the stipe and cap. Examine the cut surface with a hand lens to see if you can detect the many hyphae massed together to form the reproductive structure. Examine the gills and notice how they are produced on the lower side of the cap. Draw the sectioned mushroom in the space provided and label: **stipe, annulus** (if present), **cap, gills.**

Longitudinal section of a mushroom

Underside of mushroom cap

Cut the cap from the stipe of another mushroom (specimens may be traded in the class) and lay it with the gills up. Draw this view of a mushroom in the space provided on the previous page and label: **stipe, gills, cap.**

A Part 2 MICROSCOPIC STRUCTURE OF A MUSHROOM GILL. The gill of the mushroom is composed of compacted hyphae. Specialized hyphae become swollen at their tips to form basidia containing basidiospores.

Materials. Fresh mushrooms; microscope; forceps; slide; cover glass.

Procedure and observations. Using your forceps, lift a portion of a gill out of the cap and lay it in a drop of water on a slide. Add a cover glass and examine with low power. Brownish, oval *spores* should be visible on the gill surface. Depending on the species of mushroom you examine, the spores should be produced in groups of two or four. Bring the spores into sharp focus. Then shift the focus and examine the tips of the hyphae below the gill surface. The spore-bearing hyphae branch into the gills and end in club-shaped *basidia* at the gill surfaces. When you magnify the surface of a gill you can see the ends of basidia as rounded structures just below the spores. These are shown in Fig. 19-3-b. Compare your slide with the figure and label: spore, basidium.

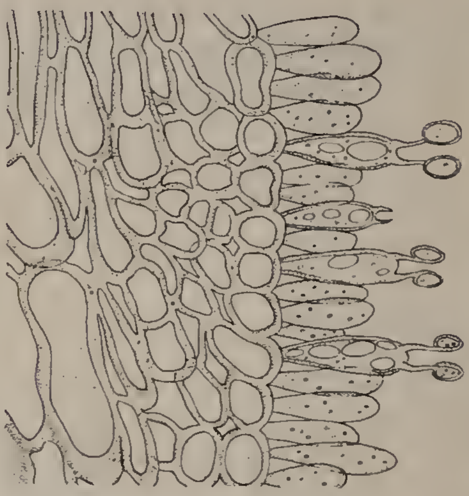


Fig. 19-3-b Surface of a mushroom gill (L.P.)

A Part 3 MUSHROOM SPORE PRINT. In addition to external structures, biologists also use the color of the spores to identify mushrooms. Since individual spores are very small, their color is usually determined by making spore prints of the mushrooms to be identified. This Part can be done as a class demonstration.

Materials. Fresh mushroom caps; white paper; glass covering; hand lens.

Procedure and observations. Remove the cap from the stipe of a fresh, well-opened mushroom. Lay it, gills down, on a piece of white paper and cover it with a dish, as shown in Fig. 19-3-c. Leave it overnight. Then, remove the cover and lift the mushroom cap from the paper carefully. The spores that dropped from the gill surfaces should leave an outline of the gills. (a) What color are the spores? .... (b) From what you have learned about the mushroom plant body, explain how mushrooms can appear in a certain place in your yard year after year without there being any sign of them between seasons. ....

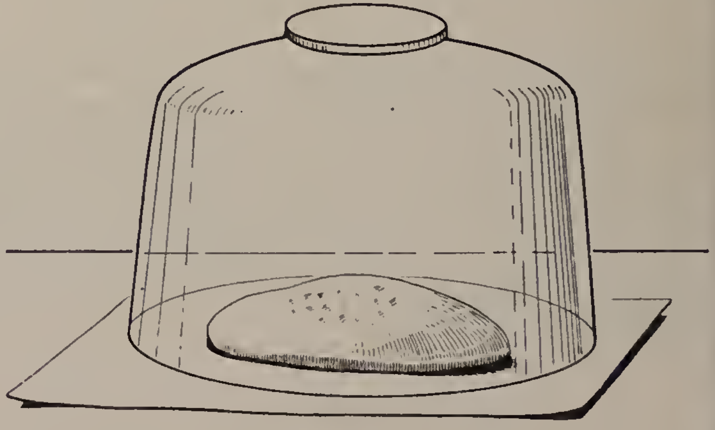


Fig. 19-3-c Mushroom spore print

....

....

The Algae

20-1

Purpose To become familiar with representatives of the various algal phyla.

The protist kingdom includes a large number of algal phyla. These are separated from one another on the basis of their cell structure, photosynthetic pigments, and stored food. No other group of plants has so many different colors or forms.

The common names that are given to the phyla of algae—blue-green, green, yellow-green, golden-brown, brown, and red—refer to the color that is usually most prominent in the cells. Color, however, is not a reliable basis for classification. In form, which is a more reliable basis, the algae may be unicellular or multicellular. The colonies may be formed of loose aggregates of cells or have a definite shape. They may form filaments or platelike growths. They may be branched or unbranched, sessile or free-floating, macroscopic or microscopic.

In your survey of the algae, you will study organisms that range from the simplest form of blue-green cells, resembling bacteria in many respects, to reds and browns which contain tissues similar to vascular plants.

A Part 1 BLUE-GREEN ALGAE—CYANOPHYTA. The blue-green algae are found mainly in fresh water, but they also occur as masses on rocks, stones, tree trunks, and damp ground as well as in salt water. They are characterized by a blue pigment, phycocyanin, which they have in addition to chlorophyll. Some genera contain a red pigment, phycoerythrin.

Materials. Cultures of *Nostoc, Anabaena, Oscillatoria,* and *Gloeocapsa (Anacystis);* microscope; slides; cover glasses; dropping pipette; dissecting needles; scissors; colored pencils; hand lens.

Procedure and observations. Examine a ball of *Nostoc* with your hand lens. (a) Describe it.

....

Cut out a section of the ball one-eighth inch square and mount it on a slide in a drop of water. Add a cover glass and tap lightly to flatten the material. Examine under low power. Notice the many curved and twisting *filaments* embedded in the jellylike *matrix* of the *Nostoc* ball. Study the filaments under high power and see if you can find swollen, empty cells called *heterocysts.* (b) Are they at the end of the filament or in the middle? ....

(c) Are they present in all the filaments? .... (d) Is more than one present in a filament? .... In the space on page 166 draw several filaments as they appear under high power. Label: matrix, heterocyst, filament.

Anabaena is another filamentous blue-green alga. The filaments of *Anabaena* are solitary and never appear in colonies. Put a small quantity of *Anabaena* culture on a slide. Spread the filaments apart and add a cover glass. Examine under low power, then high power. (e) In what ways does *Anabaena* resemble *Nostoc*? ....
Study the slide carefully to see if you can locate the enlarged oval cells in a filament. These are *spores,* which are protected by a thick wall and contain stored food. (f) Are the spores terminal or distributed through the filaments? ....
Spores separate from the filament and germinate a new cell which eventually forms a new filament through fission. (g) Do you find *heterocysts* in some of the filaments? ....
(h) How does *Anabaena* differ from *Nostoc* in structure and in method of reproduction?

....

Draw a filament of *Anabaena* in the space provided and label: spore, heterocyst.

***Nostoc* filament** **Anabaena filament**

Oscillatoria is another common blue-green alga. It is found as a floating or submerged mass of filaments in stagnant water. Different species of *Oscillatoria* vary greatly in size and range in color from bright blue-green to nearly black.

Put a small amount of *Oscillatoria* in a drop of water on a slide. Spread the filaments apart with dissecting needles and add a cover glass. Examine with low power, then high power. If your *Oscillatoria* is living, watch for a slight swaying motion, especially near the ends of the filaments. The cell on the end of the filament is called the *apical cell.* Various species of *Oscillatoria* differ in the shape of this cell. Study your slide and determine whether you have more than one species. (i) If so, how do they differ? ....

....

Study the content of the cells and see if you can locate the small dots in the protoplasm. These are grains of glycogen or related *starch.* Search along a filament for one or more areas into which the cells bulge on either side. These are called *concave cells. Oscillatoria* filaments often break at this point. Draw several filaments of *Oscillatoria* in the space provided. Label: **apical cell, starch grain, concave cell** (if present).

***Oscillatoria* filaments** ***Gloeocapsa* colony**

Gloeocapsa (Anacystis) is a primitive blue-green alga. It is often found on moist flowerpots and wet rocks. A cell of *Gloeocapsa* secretes a slimy *sheath* in which it is encased. As cells divide, each new cell secretes a sheath within the old one. Put a drop of *Gloeocapsa* culture on a slide with a cover glass. Examine under low and high power. Reduce the amount of light entering the objective and study the colony. (j) List the ways in which *Gloeocapsa* is similar to bacteria. ....

....

(k) different from bacteria. ....
Draw a colony of *Gloeocapsa* in the space provided and label: **cell, sheath.**

A Part 2 GREEN ALGAE—CHLOROPHYTA. Green algae are the most widely distributed of all the algae. They are found nearly everywhere there is moisture. They vary in complexity from single-celled organisms to many-celled branching filaments.

Materials. Cultures of *Protococcus (Phytoconis), Spirogyra* (living or preserved conjugative and vegetative forms), *Ulothrix,* and *Oedogonium;* microscope; slides; cover glasses; dropping pipette; dissecting needles; hand lens; scissors; colored pencils.

Procedure and observations. *Protococcus (Phytoconis)* is one of the most common of the unicellular forms. It can be found as a green film on damp walls, rocks, fence posts, and the bark of trees. Examine the growth of *Protococcus* on a piece of bark with a hand lens. Draw the piece of bark and shade the area covered with *Protococcus* in the space provided. Scrape some of the algae from the bark into a drop of water on a slide. Avoid getting pieces of bark on the slide. Spread the cells through the water and add a cover glass. Examine with low power and high power. Find a single cell and draw it under high power in the space provided. Label: **cell wall, chloroplast, nucleus.** Shade the chloroplast green. Examine your slide to find colonies. (a) Describe the walls between the cells that are joined. ....

....

Draw two colonies. (One may contain only two cells, the other four cells.)

Bark with *Protococcus* **—** ***Protococcus* cell (H.P.)** **—** ***Protococcus* colonies (H.P.)**

Spirogyra is a common unbranched filamentous alga which forms a green growth on the surface of quiet ponds and streams. Mount several filaments (living if possible) in a drop of water on a slide. Add a cover glass. Examine the cells under low and high power. (b) Are the cells in a filament all the same shape and size? .... (c) How many cells constitute a *Spirogyra* organism? .... Examine the spiral ribbonlike *chloroplast.* (d) Do all of the chloroplasts spiral in the same direction in the cells of a filament? .... Some species have more than one chloroplast. Examine the slide and determine whether you have more than one species. (e) If so, in what way other than the number of chloroplasts do they differ? ....

....

Locate the rounded bodies of stored food on the chloroplasts. These are the *pyrenoids*. Study several cells and count the pyrenoids. (f) Is the number uniform for each cell of a filament? .... The *cytoplasm* lies in a dense layer around the wall and extends through the cell in strands. The *nucleus* is embedded in strands of cytoplasm. The large cavities you see in the cell are *vacuoles*. Locate the gelatinous *sheath* surrounding the filament. (g) How would the sheath aid in the identification of a *Spirogyra* filament? .... Make a drawing of three cells in a filament showing how the cells are joined. Label: **sheath, cytoplasm, vacuole, chloroplast, pyrenoid,** and **nucleus.**

Look for filaments of conjugating *Spirogyra*. If you cannot find them, you may use preserved material. During conjugation, the entire content of a *minus* (−) *cell* passes through the *conjugation tube* and unites with the content of a *plus* (+) *cell*. These contents are referred to as *gametes*. The fusion body resulting from the union of two gametes is a *zygote*. Locate two filaments in the process of conjugation. (h) What two evidences indicate they are conjugating? ....

(i) Is the movement of the cell content all in one direction? .... (j) Do all the cells in the two filaments complete conjugation? .... (k) Describe the zygote. ....

....

Make a drawing in the space provided, and label: **— cell, + cell, conjugation tube, gamete,** and **zygote.**

Spirogyra cell (H.P.)

Conjugating *Spirogyra* (L.P.)

Ulothrix is a filamentous green alga whose basal cell may be modified into a *holdfast* cell. These algae may be attached to rocks or be free-floating like *Spirogyra*. Mount a few strands of *Ulothrix* in a drop of water and add a cover glass. Examine under low power and high power. Study the filament carefully. (l) Can you find the holdfast cell? ....

(m) If so, how does it differ from the other cells of the filament ? ....

(n) Describe the chloroplast. .... Make a drawing of a *Ulothrix* filament in the space provided and label: **chloroplast, nucleus,** and **pyrenoid.**

Oedogonium is a filamentous green alga which is usually attached by a holdfast cell when young, but is free-floating when mature. Mount a few strands of *Oedogonium* in a drop of water. Separate them gently with a dissecting needle and add a cover glass. Study the filament under low power and high power. (o) Are all the cells in a filament identical? ....

(p) If not, how do they differ? ....

The large rounded cells are the *oogonial* cells, which produce the egg cell. The small, short cells are the *antheridial* cells, which produce the sperm cells. (q) How does reproduction in *Oedogonium* differ from that in *Spirogyra*? ....

....

Draw a filament of *Oedogonium* in the space provided and label: **antheridium, oogonium, chloroplast, pyrenoid, vegetative cell.**

Ulothrix filament (L.P.)

Oedogonium filament (L.P.)

A Part 3 **STUDY OF DIATOMS.** The diatoms are found in fresh and salt water. They occur singly or in colonies and have a wide range of shapes and color. Some are rod-shaped; others are circular and wedge-shaped. Their cell walls have innumerable markings which are due to tiny perforations in the wall.

Materials. Culture of diatoms; microscope; slides; cover glasses.

Procedure and observations. Prepare a wet mount of the diatom culture. Examine the entire field and locate individual cells and any colonies that are present. (a) What colors do you find in the diatoms? ....

Study the walls of several of the specimens. (b) Are you able to detect the individual dots or do they appear as lines? .... Draw several of the forms and colonies of the diatoms in the space provided.

A Part 4 **STUDY OF MARINE ALGAE.** Most of the brown and red algae are marine forms. Brown algae are similar to green algae in body type and methods of reproduction. However, the brown algae have multicellular sex organs and a more complex type of vegetative body. Some of these algae resemble the vascular plants in organization. However, they lack xylem, a cuticle, and special coverings for their sex organs.

Red algae lack flagellated cells and contain a red pigment closely resembling phycoerythrin. The thallus is usually multicellular and may be simple or branched. They are usually attached by a holdfast, and are found growing in warm seas. Some red algae grow attached to other algae and some are even parasitic.

Diatoms (H.P.)

Materials. Culture of marine algae including *Fucus* and *Chondrus crispus* (fresh or preserved).

Procedure and observations. Study a specimen of *Fucus* and locate the *holdfast,* which holds the alga to the rock or other substrata. (a) How does a holdfast differ from a root? ....

.... From this holdfast, a *stipe* holds the *frond.* Examine the stipe and frond of your specimen. Note that at places along the margin of the frond, *bladders* are located so that the plant floats towards the surface when covered with water. Examine the tips of the fronds and locate the enlarged areas, or receptacles, in which gametes are formed. Draw a portion of your specimen in the space provided. Label: **holdfast, stipe, frond, bladder.**

Study a specimen of *Chondrus crispus,* or Irish moss. Note the curly margin of the fronds. Locate the tough stipe and holdfast area of the plant body. Draw a portion of your specimen in the space provided. Label: **holdfast, stipe,** and **frond.**

Fucus

Chondrus crispus

Diversity in Algae

20-2

Purpose To examine various algae collected in your region; to study seasonal growth of algae.

In your study of diversity in the algae, you will observe some of the most interesting organisms found in the protist kingdom. You will find colonies consisting of yellow or green balls with flagella which enable them to dart or roll about; green, flat plates which move in circles and yellowish brown cells which move in a zigzag manner. You will observe others that have no means of locomotion and many that grow on other algae. Some of the algae will be slimy to the touch. Many are single filaments; many are branched and some form secondary branches.

You will find that the concentration of certain kinds of algae in an area is dependent upon the environment. For example, the number of diatoms present in a lake or pond is dependent upon the amount of silicon present. The degree of acidity of the water has a definite bearing on the kinds of algae present.

B Part 1 COLLECTION, EXAMINATION, AND IDENTIFICATION OF FRESH-WATER ALGAE. Fresh-water bodies contain a large number of living organisms. The less rapid the flow of water, the more individuals you will find. You will find more greens and blue-greens in tributaries and back waters than in main streams. The characteristic algae of swiftly running water are those that have holdfast cells to anchor them to the substrata.

You will find it interesting to collect samples from several fresh-water habitats including ponds, lakes, streams, tributaries and backwaters. Although many forms may be more numerous in one habitat than another, some forms will inhabit each of the areas you explore.

Materials. Microscope; slides; cover glasses; thermometer; collecting bottles; culture dishes or battery jars; forceps; dropping pipette; dissecting needles; classification keys on algae; notebook.

Procedure and observations. Collect as many different algae as possible from various environments. Prepare a small notebook in which to record such data as collection number, place collected, depth, temperature of water, whether the plants were stationary or free floating, and nature of the habitat. Put each sample in a clean jar, and assign a collecion number to it. Transfer your samples into culture dishes or battery jars. Be sure you mark them with the collection number of the culture. Use water from the native environment, if possible.

Prepare slides of the various algae you have collected. Mount the filamentous types with forceps in a drop of water. Always separate the filaments with dissecting needles. Be sure to make slides of the sediment in the bottom of the cultures. Use a pipette to place the sediment on a slide. This sediment may contain diatoms, desmids, and other plankton forms. Compare your algae specimens with those shown in Fig. 20-2-a. If you do not find an illustration of your specimen, check in various books containing information concerning the algae. Try to use a key for identification.

Algae are identified on the basis of the arrangement of cells in colonies, size and shape of cells, form of chloroplast, and other characteristics. Study each of your specimens and record all data relating to them on the table.

You may prepare a similar chart to record information relating to other algae you collect.

Pertinent information	*Specimen number*							
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Place collected								
Nature of habitat (rapids, quiet)								
Condition of water								
Depth								
Temperature								
Stationary or free floating								
Cell grouping								
Color								
Shape of chloroplast								
Motility								
Other distinguishing characteristics								
Classification group								
Name								

Interpretation. On the basis of the data you have obtained for each specimen, discuss the distribution of algae in fresh-water environments, giving examples of adaptations to variations in the environment. ....

....

....

Explain why some forms are found in only one habitat. ....

....

C Part 2 SEASONAL GROWTH OF ALGAE.

Problem. How does temperature affect the growth of algae?

Some algae live in very cold water and even on snow; others such as the red algae live in hot springs with very high temperatures. What effect does temperature have on the growth of algae? Do slight fluctuations in temperature alter the flora of a given pond, stream, or lake? You can determine whether temperature is an important factor in the environment of algae by doing a survey of the flora of a pond at various times of the year.

Suggested materials. Collecting bottles; thermometer; microscope; slides; cover glasses; identification keys of algae; field notebook.

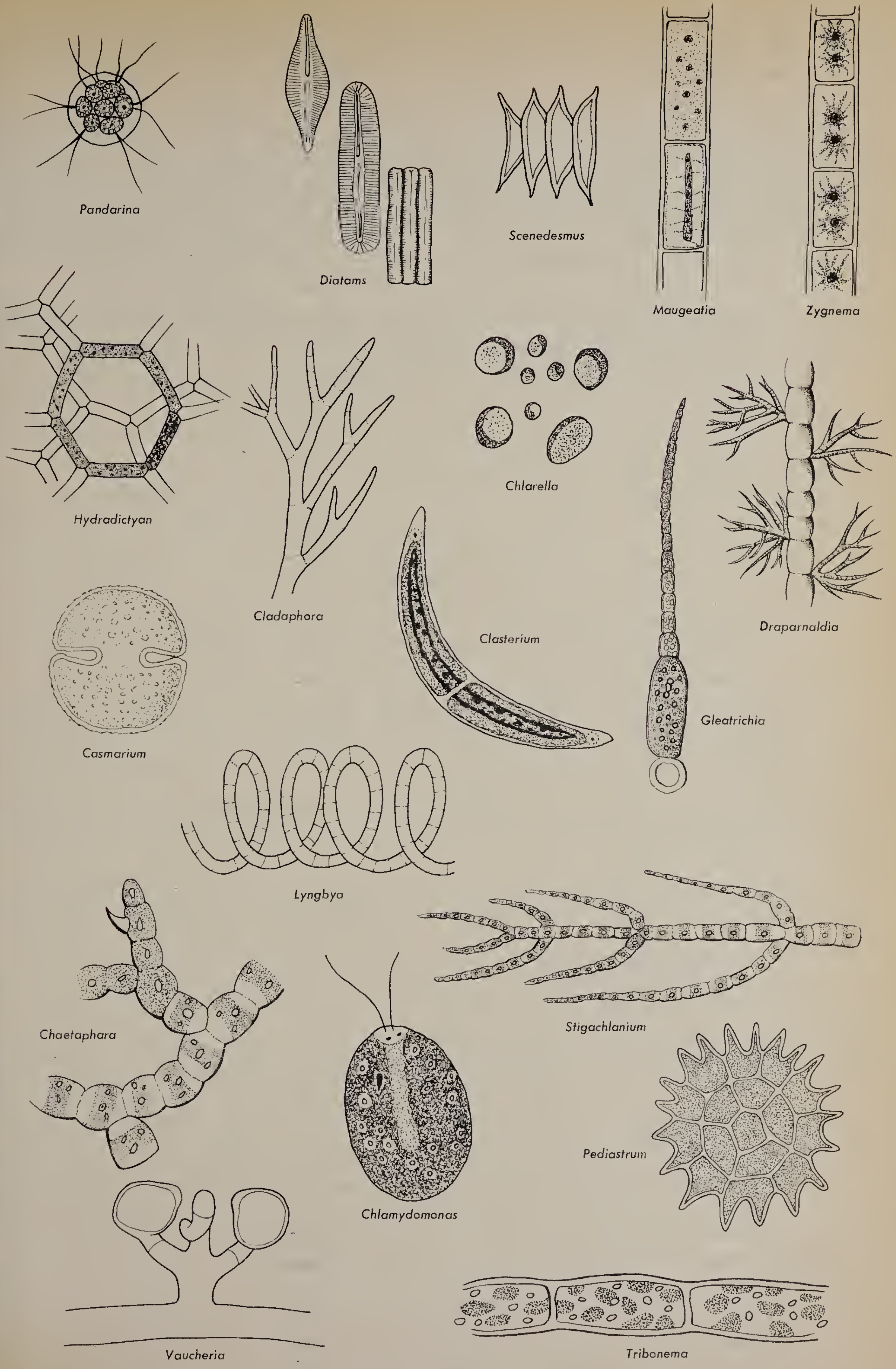

Pandarina
Diatams
Scenedesmus
Maugeatia
Zygnema
Hydradictyan
Cladaphora
Chlarella
Draparnaldia
Clasterium
Gleatrichia
Casmarium
Lyngbya
Stigachlanium
Chaetaphara
Chlamydomonas
Pediastrum
Vaucheria
Tribonema

Fig. 20-2-a Fresh-water algae

Suggested procedure. Select a pond that contains water throughout the year. Collect all the different kinds of algae you can find, placing a sample of each kind in a small bottle. Be sure to record the following data in the notebook you used for collecting fresh-water algae; temperature of water, depth from which the algae were obtained, and condition of water such as polluted, thin film of ice or frozen, and date. Take your specimens into the laboratory, prepare the slides of each kind and identify them, if possible. List the forms you cannot identify. Study them carefully to see if any are reproducing. Repeat your survey two weeks later. Continue making your examinations from the same collecting stations in the same pond for several months, if possible. Over this time you should observe several kinds of algae which are developing, maturing, reproducing, and drying out as new kinds are developing.

Analysis and conclusions. On the basis of your data, account for seasonal changes in the algal population of a fresh-water environment. Include such conditions as water temperature, length of daylight, and any other factors you think might be important.

Lichens as Examples of the Symbiotic Relationship 20-3

Purpose To study the structure of a lichen, to collect lichens, and to culture them.

Lichens may be grayish-green, white, orange, yellow, yellowish-green, brown, or black in color. There are three forms of lichens: *foliose,* which form flattened leaflike bodies, *crustose,* formed of hard, granular crusts on rocks and barks, and *fruticose,* which are composed of erect or hanging branched structures.

A Part 1 STRUCTURE OF A LICHEN. A lichen is formed by the association of Cyanophyta (blue-green algae) or Chlorophyta (green algae) with the Ascomycetes (sac fungi) or Basidiomycetes (basidium fungi). The relationship formed by the members is called *symbiosis.*

Materials. Lichen specimens; microscope; slides; cover glasses; dissecting needles.

Procedure and observations. Study the specimen you have. (a) What form does it have? .... Put a small piece of the lichen in a drop of water on a slide. Use a dissecting needle to separate the plant components and expose the algal cells and fungus filaments. (b) What is the color of the algal cells? .... (c) the shape? .... (d) What color are the fungus filaments? .... (e) Where are the algal cells located in relation to the fungus filaments? .... (f) Do the filaments appear to penetrate the algal cells? ....

B Part 2 COLLECTING AND CULTURING LICHENS. You can find lichens on rocks, tree bark, soils, and fence posts. Since lichens are able to tolerate low temperatures and periods of drought, they may also live on high mountains, in the arctic tundra, and in desert regions.

Materials. Envelopes for collecting samples; field notebook; microscope; cover glasses; dissecting needle; forceps; Petri dishes; lichen identification keys; Knop's solution; granulated agar.

Procedure and observations. Collect lichens of your region and put each sample in an envelope, assign a collection number to each one, and record such data in your field notebook as *collection number, date,* and *place collected.*

When you return to the laboratory, you can begin your examination and identification of the various samples you collected. Put a small piece of the lichen sample on a slide in a drop of water. Separate the algal cells from the fungus filaments with a dissecting needle. Put a cover glass over the specimen and examine under low power and high power. Study the algal cells and the fungal cells and use keys to identify them. Now you should be able to identify the lichen. Record the information in the following table.

Specimen number	*Place collected*	*Alga*	*Fungus*	*Name of lichen*	*Type of lichen*

The role of each member of the symbiotic relationship will become more evident after you culture them together and separately to compare their rate of growth under the same conditions. Since Knop's solution contains complete mineral requirements for growing plants, it can be used for culturing the lichen or its individual members. The directions for the preparation of Knop's solution are given in Investigation 21-1 Part 3.

Add 2 gm granulated agar (not nutrient agar) to 98 ml of Knop's solution. Stir constantly to dissolve the agar and bring the medium to a boil. Pour 3 mm of the medium into each of three sterile Petri dishes. Cover the dishes and allow to gel.

With forceps put a piece of a lichen on the medium in one Petri dish. Now put another piece of the same specimen in a watch glass containing water. Use a dissecting needle to separate the algal cells from the fungus filaments. Examine with a hand lens to locate the individual algal cells. With a dropping pipette, transfer several algal cells to the second Petri dish. Using forceps isolate some of the fungus filaments and put them on the medium of the third Petri dish. Put all three Petri dishes in a diffuse light. Examine your cultures daily for a period of three weeks and note any variation in the color or growth of the specimens. (a) What is the extent of growth of the specimens after one week? ....

(b) after two weeks? .... (c) after three weeks? ....

(d) Which specimen grew best? .... (e) Which specimen grew poorly? .... (f) Did any specimen fail to grow? ....

Interpretation. On the basis of your observations, discuss the way in which lichens illustrate symbiosis. ....

....

C Part 3 EFFECT OF LIGHT ON THE ISOLATED ALGAL CELLS, ISOLATED FUNGUS, OR ENTIRE PLANT

Problem. What is the effect of light on the isolated algal cells, isolated fungus, or the entire plant?

You have learned that light is necessary to photosynthesis, which is carried on by the algal member of the lichen. How important is light to the entire lichen? Can the alga or fungus members live in total darkness? You can determine whether light is necessary to the lichen or its members by growing them under controlled conditions.

Suggested materials. Lichen specimen; microscope; slides; cover glasses; dissecting needle; dropping pipette; forceps; Knop's solution; 6 sterile Petri dishes; granulated agar.

Suggested procedure. Using the procedure suggested in Part 2, prepare six sterile Petri dishes with Knop's solution and granulated agar. Put a portion of a lichen on the medium of two Petri dishes. With a pipette transfer isolated algal cells to the medium of two other Petri dishes. Then transfer a few isolated fungus filaments to the medium of the remaining dishes. Divide the series you prepared and put one of each specimen in the dark and one of each in a bright light. Record any changes in color or growth of the specimens.

Analysis and conclusions. Your study should enable you to determine why there are variations in the growth of the lichen, isolated algal cells, and fungus filaments under different light intensities. The study should also demonstrate the relationship of the alga and fungus in the lichen.

The Mosses

Purpose To study the gametophyte and sporophyte generations in moss plants, to culture moss protonema, and to extend the study of bryophytes into the field with a collection and identification of mosses and liverworts.

The mosses are of special interest to the biologists because they were probably the first land plants, although they have never become fully adapted to a terrestrial environment. In classification they are placed between the nonvascular algae and the vascular ferns and seed plants. The protonema stage of the moss life cycle resembles an alga. But the mosses resemble the seed plants in having an alternation of generations in which an *n* (haploid) gamete-producing sexual generation alternates with a *2n* (diploid) spore-producing generation.

A Part 1 STUDY OF MOSS GAMETOPHYTE AND SPOROPHYTE PLANTS. The familiar clumps of green moss plants are the leafy shoots, or gametophyte generation. Although the leafy shoot may appear to have a stem and roots, these are not considered as such because they lack the vascular tissues found in these organs of higher plants.

Materials. Specimens of moss leafy shoots (living or preserved, some containing mature spores, some bearing stalks and capsules); microscope; slides; cover glasses; hand lens.

Procedure and observations. Examine the leafy shoot and locate the slender, rootlike structures at the base. These are the *rhizoids.* (a) Do they appear to branch? .... Put a section of the rhizoid on a slide and prepare a wet mount. Examine it under the high power of your microscope. (b) Do you find chloroplasts? .... (c) What color is the rhizoid? .... (d) How does the rhizoid suggest a possible evolutionary relationship of mosses to certain algae? ....
Examine the stem *axis* and the numerous *leaves* it bears. (e) Describe the arrangement of leaves on the axis. .... Remove a leaf and mount it in a drop of water on a microscope slide with a cover glass. Examine with low power. (f) Describe the arrangement of the cells. ....
.... (g) How many cell layers compose the moss leaf? ....
Look for the chloroplasts. (h) How are they arranged in the cell? ....
(i) Are the cells differentiated? .... (j) Is there protoplasmic streaming in the cells? ....

Gamete-producing organs are borne at the tips of leafy shoots in a cluster of leaves resembling small flowers. Certain leafy shoots bear *archegonia,* containing eggs. Others bear *antheridia,* which produce sperm. Examine the tips of several leafy shoots with a hand lens and see if you can find these sexual reproductive organs. If possible, locate the stalked, club-shaped antheridia at the apex of the leaf stems. Remove a portion of the apex, put it on a slide, and using a dissecting needle, macerate the material to free the sperm. Put a drop of water and a cover glass over the material. Examine the slide under low and high power. (k) Do you find any sperm? .... (l) If so, describe them. ....
....

Locate the flask-shaped or long-necked archegonia. Remove and prepare a wet mount of several archegonia. Examine them under low power and locate the egg cells. Look for the modified hairlike structures, which contain chloroplasts. These aid in the retention of water around the antheridia and archegonia.

In the space provided, draw a leafy shoot somewhat enlarged (depending on the species of moss you are using.) Label: **rhizoid, axis, leaf.**

Examine a leafy shoot bearing a stalk and capsule at the tip. This is a second moss plant, known as the sporophyte generation. It consists of a slender stalk, or *seta,* which terminates in a *capsule.* (m) Why are these sporophyte stages present only on certain leafy shoots? ....

.... Examine the capsule closely with a hand lens or widefield binocular microscope. In some species a pointed hood, or *calyptra,* covers the capsule. (n) Is a calyptra present on your specimen? .... If so, remove it carefully with forceps. Turn the tip of the capsule toward you and examine it closely with a hand lens. (o) Is a tiny lid, or *operculum,* covering the end of the capsule? .... This structure falls away when the capsule ripens and dries. If it is present, remove it carefully with forceps. This should expose a ring of *teeth* through which *spores* sift when the capsule is mature.

In the space provided, draw the tip of a leafy shoot bearing a stalk and capsule, somewhat enlarged. Label: **seta and capsule.** Show a greater enlargement of the tip of the capsule with the operculum removed. Label: **teeth.**

If the capsule of your specimen is discharging spores, dust some into a drop of water on a microscope slide and add a cover glass. Examine with high power. Examine the protective wall and content of a spore closely. Draw several spores in the space provided.

Leafy shoot　　Stalk and capsule　　Capsule in detail　　Spores (H.P.)

A Part 2 **LIFE CYCLE OF A MOSS.** The gametophyte, or haploid (n), generation of the moss life cycle begins with the formation of the spores. Each spore can germinate to form a protonema from which the leafy stems are produced. The sex organs are produced at the apex of these stems. The gametophyte generation ends with the production of the gametes. The first stage of the sporophyte, or diploid ($2n$), generation is the zygote. The foot, capsule, and stalk develop from the zygote. The spores are the last cells produced by the sporophyte generation. The complete life cycle of the moss is illustrated in Fig. 21-1-a.

Materials. No materials or apparatus required.

Procedure and observations. Examine the stages in the life cycle of a moss shown in Fig. 21-1-a. The stages progress clockwise in the figure. Label each stage of the cycle and indicate whether it is a gametophyte or sporophyte stage and whether it has an *n* or *2n* chromosome content.

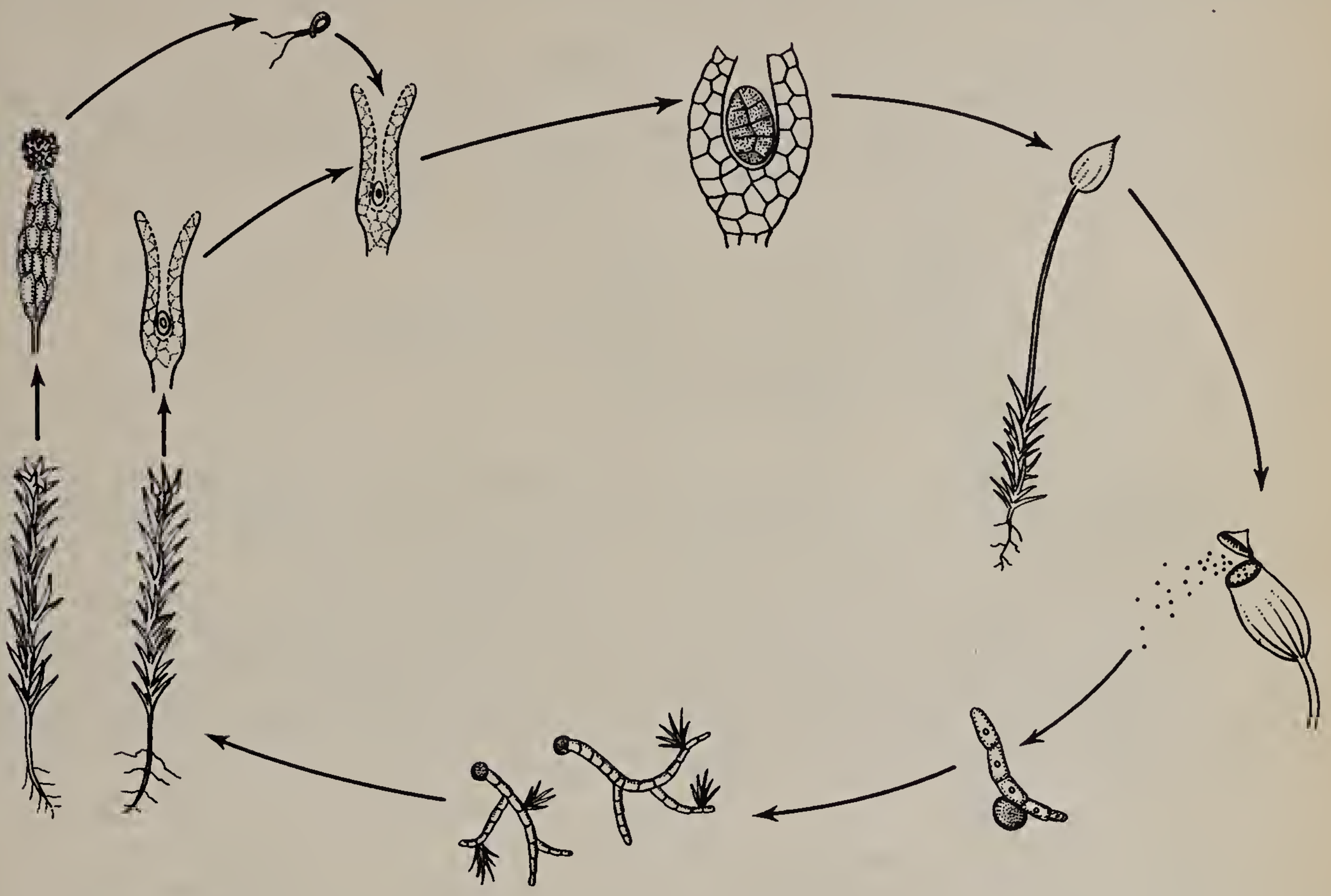


Fig. 21-1-a Life cycle of a moss

(a) In which stage may the moss travel through the air to a new environment? ....

.... (b) In what respect is the protonema of evolutionary significance in determining possible moss ancestry? ....

(c) Why does fertilization in the moss require moisture? ....

....

(d) Why is the sporophyte moss plant nutritionally dependent on the gametophyte plant?

.... (e) How does the moss life history illustrate alternation of generations? ....

....

(f) In what stage of the life cycle is the *2n* chromosome number reduced to *n*? ....

.... (g) In what stage of the life cycle is the *2n* chromosome number restored? ....

B Part 3 GROWING MOSS PROTONEMA. The protonema stage of the moss is small and difficult to distinguish in nature. However, it may be cultured in the laboratory on a special medium on which moss spores are sown. Spores germinate easily, and will quickly produce conspicuous green masses of protonema.

Materials. Spores from a fresh, ripe moss capsule; hand lens or widefield binocular microscope; Petri dishes; hot-air oven; forceps; cover glasses; slides; moist chamber; distilled water; Knop's solution (see directions in *Procedure and observations*).

Procedure and observations. Sterilize one or more Petri dishes in an oven for two hours at 350°F. The culture medium consists of Knop's solution mixed with agar to form a gel. To prepare Knop's solution, dissolve the following in 1 liter of distilled water:

0.8 gm	calcium nitrate
0.2 gm	potassium nitrate
0.2 gm	potassium dihydrogen phosphate
0.2 gm	magnesium sulfate
trace	ferric phosphate

To 98 ml of Knop's solution, add 2 gm granulated agar (not nutrient agar). Bring the medium to a boil with constant stirring to dissolve the agar. Pour the medium in a Petri dish to a depth of ¼ in (5 mm). Cover the dish and allow the medium to gel.

Sift spores from a fresh, ripe moss capsule on the surface of the medium. Cover the dish and place it in reduced light. Keep the dish closed during the culturing period to prevent moisture loss and contamination with spores of molds and other organisms. The spores should germinate and produce protonema in 10 to 14 days. Examine the culture through the cover with a hand lens or wide-field binocular microscope each day after the spores germinate. When the protonema have developed, remove one with sterile forceps and mount it in a drop of water on a microscope slide with a cover glass. Examine with low power and high power.

If possible, maintain the culture until the buds have begun to appear. When they begin to appear, remove one with sterile forceps and mount it in a drop of water on a microscope slide with a cover glass. Examine with low and high power. (a) How does it differ in structure from the protonema that produced it? ....

....

Interpretation. Explain why the buds formed by the protonema differ in structure from the protonema that produced them. ....

....

....

C Part 4 FIELD STUDY OF BRYOPHYTES

Problem. To become acquainted with the bryophytes in your region and to determine their various habitats.

The mosses are found throughout the world. Variations in life cycle, size, and color have adapted them to many different habitats. They grow on trees, rocks, ravines, shaded hillsides, and decaying stumps and logs; in loose sand, open fields, and among the leaf litter of the forest floor; and along the banks of streams.

Suggested materials. Compound microscope; hand lens (10X); a large sack or bag; small paper sacks or flat pieces of paper (5 × 8 or 8 × 10 inches); a knife with 3-inch blade; forceps; dissecting needles; dissecting microscope; slides; cover glasses; notebook; taxonomic keys to the mosses.

Suggested procedure. Since the mosses are not a conspicuous group of plants, you will have to look carefully for them. Look everywhere there is moisture and reduced light. Collect good shoots bearing stalks and capsules. Put the specimens in different paper bags, and record the location, date, and substratum in your notebook. If you cannot examine the specimens immediately, spread them out on papers, put blotters between them, and put a large book on them. When they have dried, put them in folders, being sure to identify each one. You can keep them indefinitely since they do not mold, and they are seldom eaten by insects that often destroy specimens of dried flowering plants. If you want to keep some of the specimens alive, plant them in a terrarium, where they should thrive for many months.

Use identification keys to trace your specimens to their particular genus. (If they have been previously dried, you will have to soak them first.) Leaf and capsule structure are used for identification purposes. Is the leaf broad, narrow, or tapered? Is its margin entire, toothed, or rolled? Does the leaf have a single or double midrib, or is the midrib lacking? If a midrib is present, does it extend beyond the leaf to form a bristle, extend to the tip of the leaf, or does it stop at the middle of the leaf? The shape of the leaf cells is also important. You will need to examine them under the microscope to determine whether they are similar in shape, elongated, hexagonal, or spindle-shaped.

Determine whether the capsule is straight, erect, inclined, or whether it hangs downward. Examine the surface and determine whether it is smooth or ribbed. The seta may be long or short, smooth or rough, and variously colored. The calyptra may be hood-shaped or mitrate, hairy or smooth. The size and shape of spores are often used as characteristics for identification.

Analysis and conclusions. Summarize your study in a series of drawings or photographs to show the various kinds of mosses that you were able to find in your region. From your study you should gain a better understanding of their ecology, morphology, and taxonomy.

Purpose To study the structure of the fern sporophyte and gametophyte plant bodies and alternation of generations in the life cycle of the fern.

In the more recent classifications, the ferns are classified in the same phylum as the seed plants because, like the seed plants, they have specialized vessels for conducting water. These vessels, called tracheids, permit the formation of a larger plant body and allow the plant to grow in drier situations than the moss plants. However, like the moss plant, the ferns still require water for fertilization.

A Part 1 STUDY OF THE SPOROPHYTE FERN PLANT. The familiar fern plant is the spore-producing stage. This conspicuous sporophyte generation is not attached to the gametophyte, as it is in the mosses.

Materials. Freshly collected or potted sporophyte fern plants (if available, a fresh fern frond bearing sori will be sufficient); hand lens; microscope; gooseneck lamp; slides; cover glasses; dissecting needle; glycerin.

Procedure and observations. If an entire sporophyte fern plant is available for study, remove it carefully and wash the soil from the roots. Otherwise, examine the *Polypodium* fern shown in Fig. 21-2-a. Observe the horizontal underground stem, roots, and leaves. In all but the tree ferns of the tropical rain forests, the stem, or *rhizome,* is horizontal and grows just beneath the surface of the ground. *Roots* emerge along the rhizome. Leaves, or *fronds,* grow at intervals along the rhizome. The young frond pushes through the soil as a "fiddle-neck." As it grows, the fiddle-neck unrolls. A mature frond consists of a stalk, or *seta,* and a blade that is divided into leaflets, or *pinnae.* In many ferns, the pinnae are finely divided (in a few species, the blade is entire). At maturity, certain of the fronds bear fruit dots, or *sori,* on the lower surface of the pinnae. These are the fertile fronds. A sorus consists of a cluster of *sporangia* in which *spores* are produced. (a) How does the sporophyte generation of the fern compare with the sporophyte of the moss? ....

....

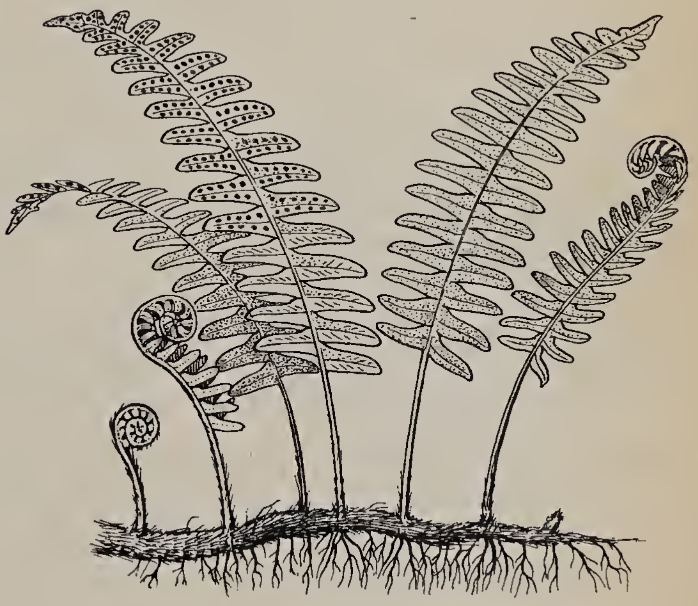


Fig. 21-2-a Sporophyte fern plant (*Polypodium*)

In Fig. 21-2-a, label: **rhizome, root, fiddle-neck, seta, pinna, sorus.**

Using a fresh fern frond bearing sori, examine a single pinna with a hand lens. Notice the structure of the blade and the location and number of sori. In many ferns, a membranous ***indusium*** covers the cluster of sporangia in a sorus. Depending on the genus and species, the indusium may be heart-shaped, circular, cup-shaped, or elongated. In some species, including the *Polypodium,* the indusium is lacking.

Using the space provided, draw a single pinna from your specimen, enlarged with the hand lens. Use care in locating the sori and showing the indusium (if present) properly. Your drawing should be sufficiently accurate to identify the genus of your specimen. Label: blade, vein, sorus, indusium (if present), sporangium.

Scrape several sporangia from a sorus of your fern into a drop of water on a slide. Add a cover glass and examine under low power of your microscope. Notice that the sporangia resemble a helmet from a suit of armor. Find the *stalk* attaching the sporangium and the thin-walled *lip cells* on one side of the sporangium. Notice the cells of the *annulus,* which extend around the sporangium from the lip cells. These cells have thick walls on three sides. *Spores* should be visible within the sporangium.

Draw the sporangium in the space provided, using care in showing the various cells composing it. Include the correct number of spores visible in the sporangium. Label: stalk, lip cells, annulus, spore.

Pinna showing sori (enlarged)

Sporangium (L.P.)

Sporangium discharging spores (L.P.)

When you have completed your drawing of a sporangium mounted in water, put a drop of glycerin at the edge of the cover glass. Watch for a change in the sporangium. If no change occurs, add additional glycerin, one drop at a time, until the sporangium opens. (b) Where did the cells separate as the sporangium opened? ....

(c) Describe the change in the cells of the annulus. ....

....

(d) How did the glycerin cause this change? ....

....

(e) What condition in nature would cause the sporangia to burst open and discharge spores?

....

Draw an open sporangium and spores discharged into the glycerin in the space provided. Show accurately the change in shape of the cells during bursting of the sporangium sac.

You may want to try a variation of this experiment, using heat from a lamp to open the sporangia. Dust some sporangia onto a dry slide and examine with low power of your microscope. Put a gooseneck lamp near the stage with the light directed onto the slide. With sufficient heat, the sporangia will burst open and discharge spores.

Examine a prepared cross section of a fern rhizome under low power and high power. The arrangement of tissues will depend on the species of fern you examine. Find the ends of rods or strands of *vascular tissues* extending through the rhizome. *Xylem* and *phloem* cells compose these vascular tissues. Thin-walled *pith* cells compose much of the rhizome. An *epidermis* lies on the surface. (f) Describe the arrangement of vascular tissues and any other tissues you can identify in your section of a fern rhizome. ....

....

....

A Part 2 **STUDY OF THE FERN GAMETOPHYTE PLANT.** The prothallus is the gametophyte plant body. It is seldom noticed in nature because it is small and inconspicuous (about ¼ in. in diameter). In this Part you will use prepared slides to study the underside of the prothallus. If living material is available, you will use it to become more familiar with the gametophyte fern plant.

Materials. Prepared slides of fern prothallus; living prothalli (if available); microscope; slides; cover glass; dissecting needle.

Procedure and observations. Examine a prepared slide of an entire fern *prothallus,* or refer to Fig. 21-2-b. Notice the *rhizoids* at the lower end. (a) How are they different from the roots of the sporophyte fern plant? ....

....

Numerous dome-shaped *antheridia* situated among the rhizoids bear sperm. Flask-shaped *archegonia,* situated below the *apical notch* of the prothallus, contain eggs. (b) How does a sperm travel from an antheridium to an archegonium during fertilization? ....

....

(c) Is the fern prothallus as well suited to a land environment as the sporophyte? Explain your answer. ....

....

....

....

(d) Why is the prothallus limited in the size it can attain?

....

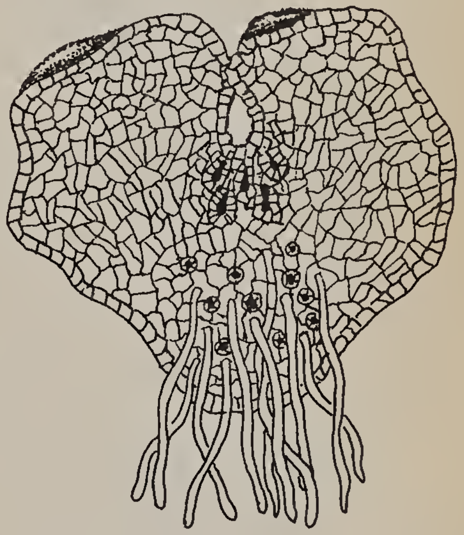


Fig. 21-2-b Fern prothallus

In Fig. 21-2-b, label: **rhizoid, antheridium, archegonium,** apical **notch.**

If living fern prothalli are available, prepare a wet mount and examine the ventral (lower) surface under low power of the microscope. (e) Describe the cellular makeup. ....

.... Look for the antheridia, which are located on the basal region of the prothallus. Using a dissecting needle, macerate an antheridium and look for the very small sperm cells. (f) Describe the sperm cells. ....

....

(g) Are any of them moving? .... Look for the archegonia, which are located near the central notch. Try to find the egg cell in the basal portion of the archegonium.

A Part 3 **LIFE CYCLE OF A FERN.** The life cycle of the fern shows how this plant lies between the mosses and the seed plants in evolutionary development. Although the ferns are terrestrial, they are still dependent on moisture for reproduction. There is an alternation of generations in which the sporophyte is independent and more conspicuous than it is in the mosses. The ferns continue the trend toward a smaller, dependent gametophyte.

Materials. No materials or apparatus required.

Procedure and observations. The various stages of the fern life cycle are shown in Fig. 21-2-c. Label each stage and indicate whether it has the n *or* $2n$ chromosome number. (a) In which stage is the chromosome number reduced from $2n$ to n? .... (b) Where is the $2n$ number restored? ....

(c) What is the conspicuous structure of the gametophyte generation? ....

....

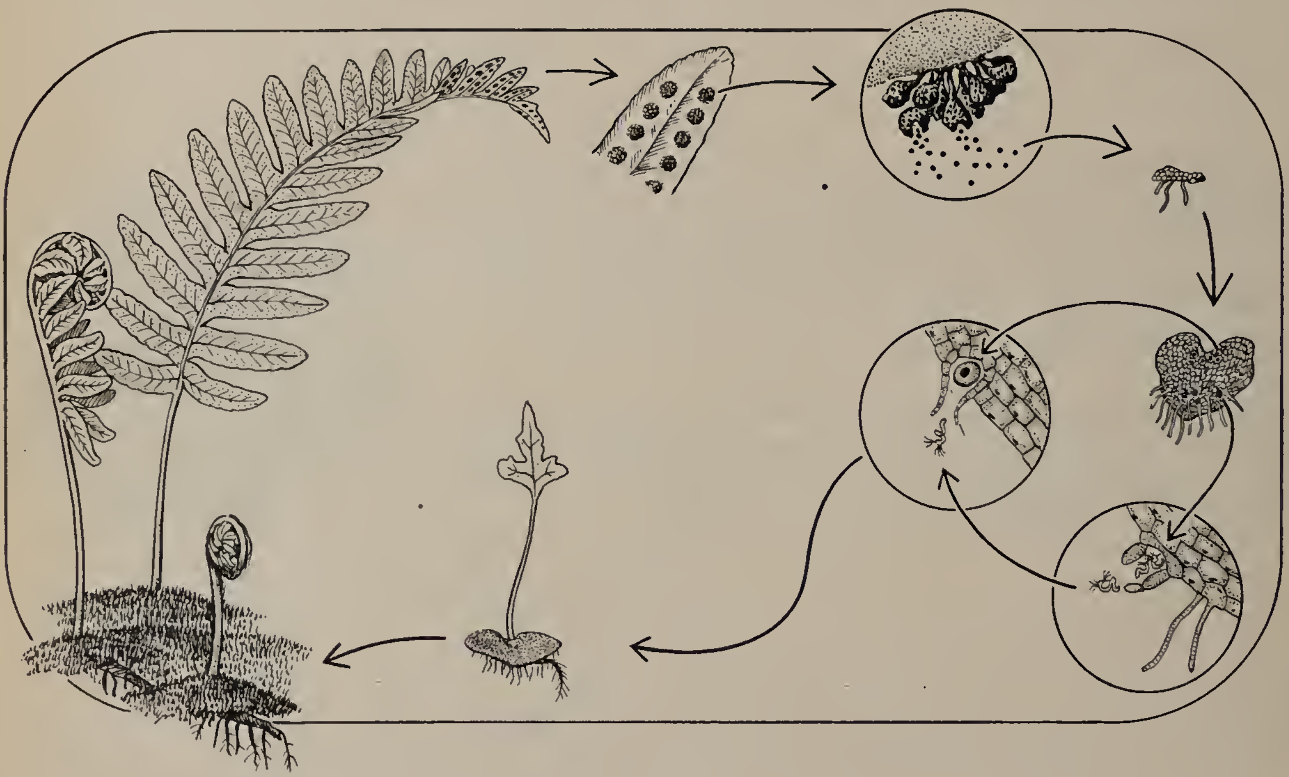


Fig. 21-2-c Life cycle of a fern

B Part 4 **GROWING FERN PROTHALLI.** Fern prothalli may be cultured in the laboratory without difficulty, although patience is required because the process may take up to three months.

Materials. Fern frond with mature spores; humus or rich loam; shallow pan; hot-air oven; 4-5 in. flowerpot saucer; bell jar; covered box; dropping pipette; Petri dishes; glass culture dishes.

Procedure and observations. Put a cupful of humus or rich loam in a shallow pan and spread it in a thin layer. Heat it in an oven at 350°F for two hours to sterilize the soil and prevent the growth of molds and other fungi. When the soil has cooled, spread a thin layer ¼-½ in. thick) in a clean 4-5 in. flowerpot saucer. Moisten the humus and pour off any excess water. Cover the saucer with a bell jar. Put a fern frond with mature spores in a covered box and dry it several days. Shake the frond to dislodge spores. Collect spores with a dropping pipette along the edges and in the corners of the box and sow them on the soil in the flowerpot saucer. Set the saucer in indirect light and replace the bell jar. Keep the soil moist while the spores are germinating and the prothalli are growing. The spores should germinate in ten days to two weeks and produce a filamentous prothallus. However, the prothalli will not mature for two to three months. During this time the soil must be kept moist, but not wet. Petri dishes and glass culture dishes (moist chambers) are also suitable for culturing fern prothalli.

Interpretation. From your observations, explain how the germinating prothallus resembles certain green algae. ....

....

C Part 5 FIELD STUDY OF FERNS

Problem. To become acquainted with the non-seed-bearing vascular plants of your region and to determine the various habitats in which they live.

Ferns, like mosses, are distributed throughout the world and in many habitats. They are found in woodlands, moist meadows, along roadsides, railroads, and stream banks. They vary in size from tiny, floating ferns to the giant tropical tree ferns.

Suggested materials. Large book or magazine for collecting fern fronds; hand lens; field notebook; taxonomic key to the ferns.

Suggested procedure. Before you begin your collection of ferns, you should become familiar with the characteristics used in their identification. These include: general form and structure, distribution of the sori, the sporangia, and the indusia, and the form of the frond.

Collect a portion of a frond (be sure to include the fertile as well as sterile ones) and put it in your book or magazine. Assign a collection number to the specimen and record this number in your field notebook along with other data such as the location, date, and substratum.

When you return to the laboratory, identify your specimens. These can be pressed and saved for later study by drying them between several layers of newspaper.

Analysis and conclusions. Your study of the taxonomy of the ferns should further demonstrate to you the importance of structural characteristics for identification of organisms. You should also be able to form some definite conclusions as to why the ferns have become an insignificant part of the flora, and why they are unable to compete with the more highly developed flowering plants.

Root Structure 23-1

Purpose To examine the cells and tissues of a mature root and to identify the regions of a root after secondary thickening.

The main function of the root is the absorption of water and minerals. Roots also function in anchoring the plant, in storing food, and in conduction. This organ has specialized tissues for each of these functions.

A Part 1 PRIMARY TISSUES OF A MATURE ROOT. The primary tissues develop from the meristematic region at the tip of the young root.

Materials. Prepared slide, cross section of a mature root (buttercup); microscope.

Procedure and observations. Examine a prepared slide of a cross section of a mature root under low power and high power. (a) How many cells thick is the epidermis? .... Count the number of cortex cells from the epidermis in to the central cylinder on two opposite sides of the root. (b) What is the average cell thickness of the cortex? ....

(c) Are intercellular spaces present in the cortex of your section? ....

(d) How many cells thick are the endodermis and the pericycle? ....

(e) What two conducting tissues lie in the central cylinder? ....

.... (f) Locate the cambium in relation to the phloem and the xylem.

....

Make a wedge-shaped drawing from your slide in the space provided and label each of the following tissues: **epidermis, cortex, endodermis, pericycle, phloem, cambium, xylem.**

Cross section of a mature root

A Part 2 **REGIONS OF A MATURE ROOT (CARROT).** Many roots, such as the carrot and beet and perennial roots of trees, increase in diameter because of secondary thickening.

Materials. Fresh carrot; scapel or knife.

Procedure and observations. Select a small carrot and examine the outside of *secondary roots* (represented in carrots sold in stores by small knobs, because the secondary roots either dropped off or fell off in the washing process). Find the *crown,* which is the point where the stem joined the top of the root and from which the leaves arose. Draw an external outline of the carrot in the space provided and label: secondary root and crown. Next slice the root lengthwise through the center, since that region contains one of the important tissues of this organ. Draw a longitudinal section of the carrot, using your textbook to identify the tissues, and label each of these. Cut across the middle of another carrot and make an outline drawing of this cross section, using your textbook for reference. Identify all tissues and label each.

External outline of carrot   Longitudinal section   Cross section

State briefly the function of each of the following tissues present in a root before or after maturation.

1. Embryonic tissue ....
2. Epidermis ....
3. Cortex ....
4. Endodermis ....
5. Pericycle ....
6. Phloem ....
7. Vascular cambium ....
8. Xylem ....

Purpose To demonstrate water passage through the tissues of a root and the rate of mineral absorption.

One of the principal functions of a root is absorption of water and minerals from the soil. Why does water normally enter the root and pass to the tissues of the central cylinder where it is conducted to the stem and leaves? Could a condition in the soil reverse this movement and cause a plant to lose water? Do minerals enter a root independently of water movement? How rapidly are minerals absorbed by a root and conducted to other organs of a plant? These are questions of great importance in the study of plant physiology. The demonstrations in the Parts of this Investigation will provide some of the answers.

A Part 1 ABSORPTION OF WATER BY A ROOT. In Investigation 5-2, you observed the passage of water through a differentially permeable membrane covering the end of a thistle tube or lining the shell of an egg. We referred to this water passage as osmosis. The movement, you will recall, was from a region of greater water concentration to an area of lesser concentration. In this Part you will demonstrate a similar water movement through the tissues of a root.

Materials. Battery jar; ring stand; 2 tubing clamps; small-bore glass tubing, 2 feet or more long; cork borer or small vegetable corer; 1-hole rubber stopper to fit core; paraffin; molasses syrup; shallow pan (for melting paraffin); hot plate (or Bunsen burner); wire gauze; tripod.

Procedure and observations. Wet the rubber stopper and the end of the glass tubing and *carefully* insert the tube through the stopper. Select a large, straight carrot. Using a cork borer or a small apple corer, remove the core about ½-¾ in. in diameter from the center of the carrot. Make the core as deep as possible, using care not to pierce the side of the carrot. After removing the core, hold the carrot upright and fill the cavity almost to the top with molasses syrup. Then insert the stopper and glass tube firmly, being careful not to split the end of the carrot. Rest the base of the carrot on the bottom of a battery jar placed on a ring stand and clamp the glass tube securely as shown in Fig. 23-2-a. Melt some paraffin in a pan and seal around the top of the carrot with several layers applied with a small brush. Fill the battery jar with water to a level above the top of the carrot. Mark the level of the molasses syrup at the start (if it is visible), and each day the experiment runs.

(a) How far did the molasses solution rise in the tube during the first 24 hours? ....

(b) in 48 hours? .... (c) When did the molasses solution reach its highest level? ....

.... (d) How does the demonstration illustrate osmosis? ....

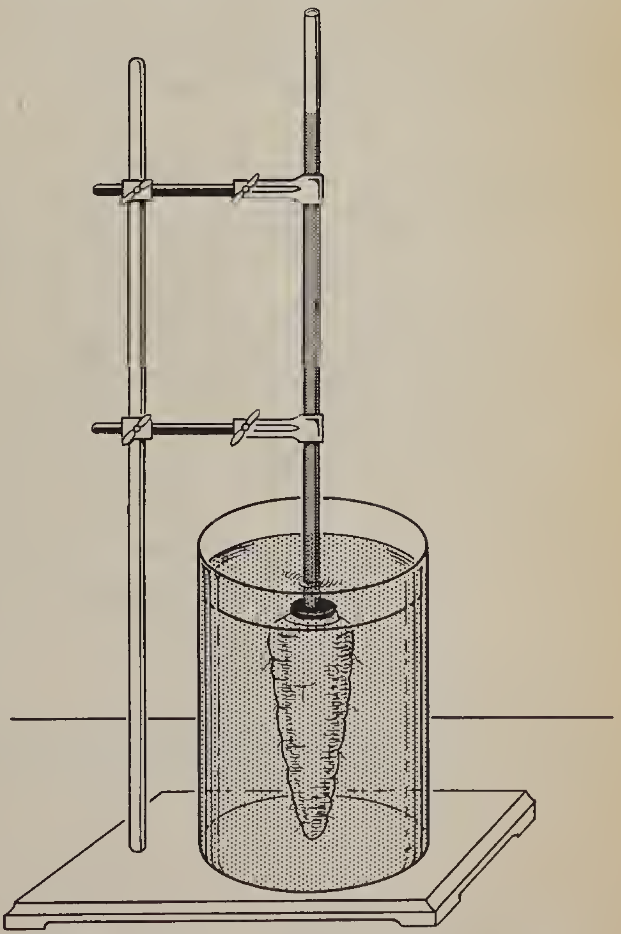


Fig. 23-2-a Osmosis demonstration using a carrot

....

.... (e) Why was the carrot bored and filled with molasses syrup? ....

....

The molasses syrup in the core is many cells removed from the surface of the carrot, yet it undoubtedly influenced the movement of water into the carrot. (f) Explain. ....

....

The molasses solution stopped rising in the tube even though the concentration of water molecules in the carrot and in the battery jar was never equalized. (g) Account for this.

....

....

B Part 2 EFFECT OF SALT SOLUTIONS ON OSMOSIS IN ROOTS. Even though soil water contains soluble minerals and other substances, a root will usually absorb water by osmosis. This will continue as long as the concentration of water molecules in the soil solution is greater than that within the cells of the root. What will happen if the greater concentration of water molecules is in the root cells rather than in the external soil solution? Then the water movement will be from the root to the soil, resulting in a shrinking or collapse of the root cells, in the process called plasmolysis. This may happen if strong fertilizers, salts, and other soluble materials form highly concentrated (hypertonic) solutions in the soil environment of a root. In this Part, you will determine the concentration of a salt solution that will cause plasmolysis to occur in a root.

Materials. Six 250-ml flasks; 6 coleus plants; salt solutions (100 ml of each) 0.1%, 0.5%, 1.0%, 5.0%, and 10.0%; glass-marking pencil.

Procedure and observations. Identify five 250-ml flasks with the five salt solutions to be used and a sixth flask as the control. Put 100 ml of each salt solution into the appropriate flask and plain water into the control flask. Select six healthy coleus plants and remove all of the soil from the roots by washing them gently with tap water. Be careful to avoid injury to the small roots and root hairs. Put a coleus plant in each flask and observe any changes which occur for 30 minutes or longer. Watch for signs of wilting of the roots and other plant parts, including the leaves. Record the results of your first observation (30 minutes) in the table. Continue the experiment for 24 hours and record changes which have occurred in this time interval.

	Plain water	0.1% *NaCl*	0.5% *NaCl*	1.0% *NaCl*	5.0% *NaCl*	10.0% *NaCl*
30-minute observation						
24-hour observation						

Interpretation. Based on your table of observations, discuss the results of your experiment. Include such information as the rate at which plasmolysis occurred, the concentration of salt necessary to cause plasmolysis, and the extent of water loss in the various plants in a 24-hour period. ....

....

....

C Part 3 ABSORPTION AND TRANSLOCATION OF RADIOACTIVE PHOSPHORUS (P^{32}) IN TOMATO PLANTS

Problem. How is phosphorus absorbed and translocated in a green plant?

In recent years biologists have used "tagged" atoms to study absorption and translocation in plants. These radioactive isotopes give off radiations that can be detected by a Geiger counter or photography.

Radioactive materials must be handled with care. This Part should be done under the supervision of someone who has had a course in radiation biology and is thus familiar with the techniques for experimenting with radioisotopes.

Suggested materials. Two tomato plants (5-6 in.); two 250-ml flasks; 10 μc P^{32}; Geiger counter; aluminum foil to wrap one flask; 1 piece of lead plate (4 × 4 in. with 1-cm opening); Saran wrap; X-ray film and developer; 2 pieces of cardboard; 2 pieces of black paper; paper to print radioautograph (glossy, high-contrast).

Suggested procedure. Select two healthy potted tomato plants, 5 or 6 inches high. Remove all the soil from the roots by washing them gently under tap water. Use the Geiger counter and test all parts of the plants for radioactivity. Put sufficient water into two 200-ml flasks to cover the roots of the plants. Add 10 μc of radioactive phosphorus to the tap water in one flask. Label this flask as *experimental.* Do not add anything to the second flask of water. Label this flask as *control.* Use the Geiger counter and determine the count per minute in the experimental solution. Put the two plants in their respective flasks. Cover the experimental flask with aluminum foil. Measure the radioactivity in the leaves at 10-minute intervals. Use a lead shield with 1-cm opening for counting. You can then calculate specific activity per square centimeter of leaf surface. Continue the counts for one hour, always beginning with the lower leaves. Prepare a line graph of the results for each set of leaves, showing time on the horizontal axis and count per minute for square centimeter of leaf surface on the vertical axis.

If you have a high count of 5,000 cpm or more, you may make a radioautograph using a 15 to 20 minute exposure. After 48 hours remove the experimental plant from the radioactive solution and wash it to free it from any radioactive material that might adhere to it. Allow the plant to dry. Putting the dry plant on Saran wrap, arrange the leaves so they do not overlap and wrap the plant to prevent its coming in contact with the film. Put the film and plant between lightweight cardboard wrapped with black paper and put in a dark place. Put several books on top of this to provide pressure so that the leaf is pressed against the film. Leave the setup for 20 minutes, then develop the film in an X-ray developer for five minutes. Use glossy high-contrast paper for printing.

Analysis and conclusions. Your data summarized in the graph and the results of your radioautograph should lead to a discussion of the absorption and translocation of phosphorus in the green plant.

Phototropism and Geotropism in Plants 23-3

Purpose To determine the effect of light and gravity on plant growth.

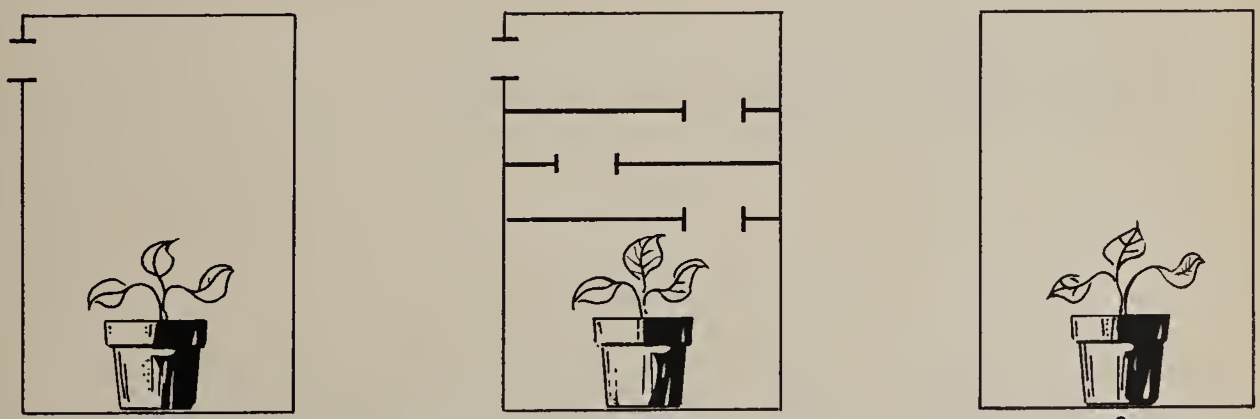
As a living organism, the green plant responds to various stimuli in its environment. A plant's responses are growth responses and are called tropisms, after the Greek word *tropos*, meaning "turn."

B Part 1 PHOTOTROPISM. The growth response of a plant to light is called phototropism.

Materials. Three potted bean seedlings; 3 shoe boxes; paper cups for planting the bean seeds; vermiculite; cardboard; potting soil; three 4-in. flower pots.

Procedure and observations. In preparation for this Part, grow several bean seeds in moist vermiculite until they have reached a height of three inches. Then transfer them to individual paper cups.

Cut out a two-inch square at the top of one of the boxes, as shown in Fig. 23-3-a. Put opaque shelves in a second box and cut two-inch squares in the shelves and at the top, as shown in Fig. 23-3-a. The third box will serve as a control. Put a paper cup containing an individual bean plant in each of the boxes. Remove the cover only for watering and observation of the plants. Put the boxes on a shelf in your laboratory so that they receive the same amount of light. Keep a record of the color of the leaves, diameter of the stem, and appearance of leaf buds.



1 Box with one opening

2 Box with shelves and one opening

3 Control box (no openings)

Fig. 23-3-a Apparatus for demonstrating phototropism

(a) Describe the growth of the leaves of the plant in box 1. ....

.... (b) in box 2. ....

.... (c) in box 3. ....

(d) Explain the variations in the leaves of the three plants. ....

....

(e) Describe the growth of the stem of the plant in box 1. ....

.... (f) in box 2. ....

....

(g) in box 3. ....

(h) Account for the differences in growth of the three plants. ....

....

For a second experiment in phototropism, select three healthy bean seedlings (about 4 in. high) and place them in the 4-in. flowerpots. Put the pots on a shelf so that the three plants receive light from one side only. Identify the plants as *1, 2,* and *3* respectively. Water the plants as needed.

Observe the plants daily for one week. (i) Describe any changes that take place. ....

....

After one week, rotate plant 1 a fourth turn; rotate plant 2 a half turn; leave plant 3 in the same position. Observe the plants for an additional week. (j) Describe what changes occurred in plant 1. .... (k) in plant 2. .... (l) in plant 3. ....

....

Now rotate plant 1 another fourth turn; rotate plant 2 a half turn; leave plant 3 in the same position. Observe the plants for another week. (m) Describe the reactions of plant 1. .... (n) of plant 2. .... (o) of plant 3. ....

.... After the third week, rotate plant 1 another fourth turn. Rotate plant 2 another half turn and leave plant 3 in the same position. (p) What did plant 3 become after the first week of the experiment? ....

Observe the changes in the three plants for another week. (q) Discuss the changes in plant 1.

....

(r) in plant 2. .... (s) in plant 3.

....

Interpretation. On the basis of these two experiments discuss the effect of light on the growth of plants. ....

....

....

....

B Part 2 GEOTROPISM. The response of a plant to gravity is called geotropism.

Materials. Three potted coleus or geranium plants; bean and corn seeds; wire gauze; string or fine wire; 2 ring stands; 3 clamps; record turntable or platform rotator (speed about 78 rpm); box 12 in. by 4 in. with opening in center to fit spindle of turntable or platform rotator; 3 straight coleus plants, might include periwinkle and a member of the grass family; 2 pieces of clear glass 6 in. by 12 in.; shallow pan to hold water in which to set the glass; rubber bands to hold the glass together; blotter paper.

Procedure and observations. Cut gauze squares large enough to cover the top of the flowerpot. Cut a slit to the center and place it around the stem and over the top of the pot. Now bring the gauze down over the lip of the flowerpot and secure it with string or fine wire. The soil should now stay in the flowerpot even if turned upside down.

Clamp one of the flowerpots upside down and another on its side, as shown in Fig. 23-3-b. Let a third plant remain upright. The plants may be carefully watered every day through the bottom of the flowerpot, but do *not* move them. (a) Why? ....

....

Observe the experiment daily and sketch the changes that occur in the space provided.

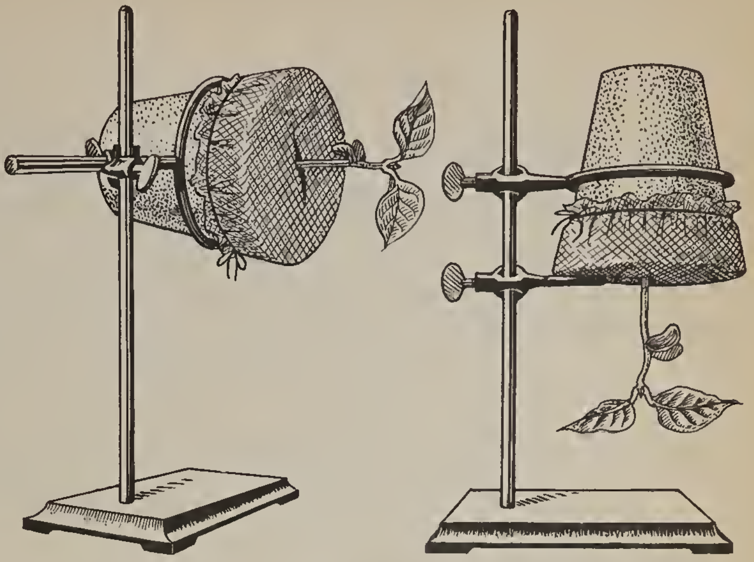


Fig. 23-3-b Apparatus for geotropism

Changes illustrating geotropism

(b) What is the first noticeable change in the plants? ....

After a week, carefully remove the plants from the pots and examine the roots. (c) What evidence of a tropism do you find? ....

.... (d) Discuss the effect of geotropism on roots. ....

....

(e) on stems. ....

....

A second experiment in geotropism can involve the germinating seed. Cut a piece of blotter paper 6 in. by 12 in. and put several seeds on the blotter, as shown in Fig. 23-3-c. Use both monocot (corn) and dicot (bean) seeds. Place the blotter between two pieces of glass. Rubber bands may be used to hold the

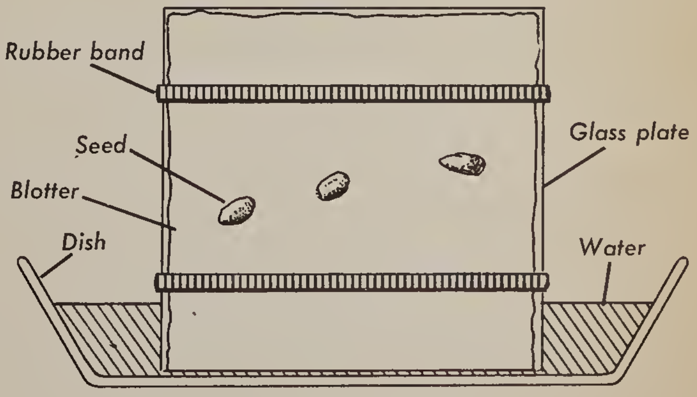


Fig. 23-3-c Apparatus to show the effect of gravity on seeds

glass together. Place the plates on edge in a pan containing water. Observe the seeds for several days. (f) Discuss the curvature of the roots of the various seeds.

A third experiment in geotropism can be done by using well-developed plants and a turntable or platform rotator. Secure a record turntable or platform rotator (speed about 78 rpm). Construct a box 12 in. by 4 in. with an opening on the base to fit the spindle on the turntable or platform rotator. Fill the box with vermiculite. Select three healthy straight plants and place one at either end of the box and the third one in the exact center. Put the box on the turntable or platform rotator, as shown in Fig. 23-3-d. Set the apparatus in a room with an overhead light source only. This light should be kept on for the duration of the experiment. (g) Explain why the light should be overhead only.

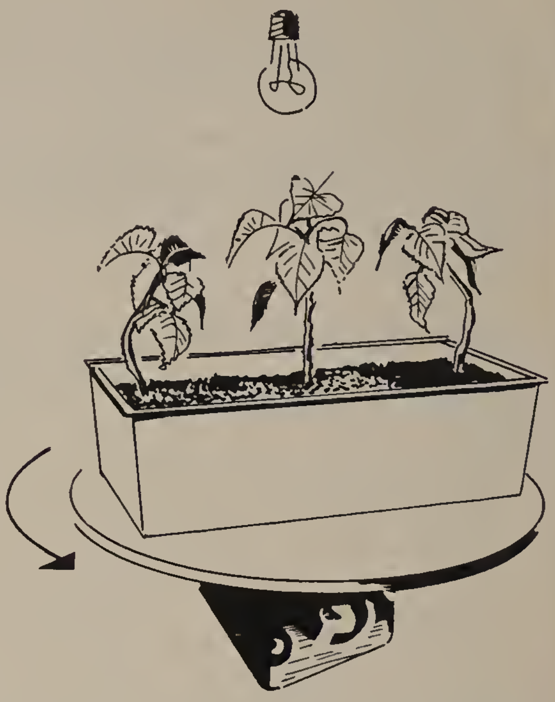


Fig. 23-3-d Apparatus for experiment in Part 2, showing periwinkle after 24 hours

Connect the turntable and allow to run for 24 hours. At the end of this time, stop the turntable, water the plants, and observe them. Record the changes in the three plants. Connect the turntable and continue the experiment for two more days. Be sure to examine the plant at the end of the 48-hour period and record your data. (h) Discuss and explain the behavior of the three plants at the end of 72 hours.

(i) In addition to gravity, what other force influenced the growth of the plants?

Interpretation. Explain the changes that occurred during the turntable experiment.

Growth Stimulators in Plants 23-4

Purpose To demonstrate the effect of various hormones and vitamins on the growth of plants.

What factors are responsible for growth and development in plants? Biologists have found that in addition to environmental factors, growth in plants is regulated by chemical substances called plant hormones. These are organic compounds produced by the plant, which, although they are produced in small amounts, influence growth. Furthermore, they are transported from the region in which they are produced to other parts of the plant body where they influence growth and other physiological activities. Thus, growth in one organ of a plant may be de-pedent upon the cellular activities of another.

The most extensively studied of plant hormones are the auxins. These accelerate growth by stimulating cell elongation. Several auxins have been isolated from germinating seeds, seedlings, and developing shoots. Auxins also inhibit growth of certain tissues. Thus, they have been used experimentally to prolong the dormant period of potato tubers and to control the fall of fruit such as apples and pears before ripening occurs.

B Part 1 EFFECT OF PLANT HORMONES ON THE GROWTH OF ROOTS. Biologists have found that auxins influence some twenty different activities in plants. In this Part you will use three substances which have been prepared commercially to promote growth in the roots of plants.

Materials. Root-forming hormone powder, indolebutyric acid crystals or prepared crystals; root-forming hormone salve; twelve 3-4 in. flowerpots; equal parts of sand and loam to put in pots; 12 vigorous shoots of coleus, begonia, or geranium; vigorous potted plant with a soft stem (coleus, begonia, fuchsia, or geranium); lanolin.

Procedure and observations. Section 1 *The effect of hormone powder on the formation of roots.* Prepare a mixture of equal parts of sand and loam and fill six 3-4 in. flowerpots (boxes or cardboard containers may be substituted). Make six cuttings 4-6 in. long of vigorous shoots of coleus, begonia, or geranium. All six cuttings must be of the same species and should be of equal length. Dip three of the cuttings into root-forming hormone powder to a depth of one inch. The other three cuttings are left untreated and will serve as controls.

Plant all six cuttings in individual pots and water well. Set the cuttings in a light place and water them as required during the experiment. Give all six potted cuttings identical treatment during the growth period. After three weeks, remove one of the experimental (treated) plants and one of the control (untreated) plants from the soil, using care not to damage the roots. If neither plant has produced roots, replace

Cutting treated with hormone powder

Cutting untreated

the cuttings and continue the experiment one or two additional weeks. When roots have formed, remove all of the cuttings and wash the soil from the roots. Compare the root growth of the experimental and control cuttings. Select one of each and record your observations as drawings in the space provided.

(a) How does the experiment demonstrate the stimulation of root growth by hormone powder? .... (b) Why were three plants used in each set in the experiment? ....

(c) Why was a control necessary? ....

.... (d) Why was it necessary to use cuttings of the same species and grow them under identical conditions? ....

....

Section 2 *The effect of indolebutyric acid solution on the formation of roots.* Indolebutyric acid is the trade name used by biological supply houses for a hormone solution that can induce root growth on stem cuttings. Repeat the procedure in Section 1 of this Part, but substitute an indolebutyric acid solution for the root-forming hormone powder. Before planting the shoots, immerse their cut ends in the solution for four to eight hours.

Summarize the results of your experiment in drawings showing root development in the experimental and control cuttings, or in a table, in the space provided. Identify each cutting.

Effect of indolebutyric acid on root development

Section 3 *Use of a root-forming hormone salve.* Hormone salves are prepared by mixing a hormone powder with a fatty substance such as lanolin. These are best obtained, prepared for use, from a biological supply house.

About half-way up the stem of your potted plant, apply the root-forming hormone salve in a ring about one centimeter (⅜ in.) wide. Observe the treated area daily for two or three weeks. (e) Was a callus formed in the treated area of your plant? .... (f) If a callus formed, how soon after treatment did it appear? .... (g) Did roots develop from the callus? .... When the roots are well developed, the stem may be cut below the callus and planted. (h) Why was it necessary to use a plant with a soft (herbaceous) stem rather than a wood plant with bark? ....

Interpretation. Summarize your observations on the effects of hormones on root growth.

....

....

B Part 2 STUDY OF APICAL DOMINANCE. In some stems production of auxin by cells of the terminal bud inhibits the growth of lateral buds. We shall study such apical dominance in the potato, which is a modified stem, and similar dominance in *Bryophyllum* leaves.

Materials. Six white potatoes; 1 potted *Bryophyllum* plant; scalpel; scissors; tray or box; shallow pan; sheet of glass; sand to put in pan.

Procedure and observations. Locate the terminal eye on each potato. Prepare three of the potatoes as shown in Fig. 23-4-a, using a scalpel to cut the groove. Put all six potatoes in a tray or box, terminal bud up, and store them in a warm, dark place. Examine the potatoes every few days until sprouting has occurred. This should require two or three weeks.

(a) Did the terminal bud develop in all of the potatoes? .... (b) Compare the growth of the buds above the groove and below the groove in the treated potatoes. .... (c) Describe the bud development of the untreated potatoes. ....

....

(d) How did the groove in the treated potatoes affect auxin conduction, as indicated by bud development? ....

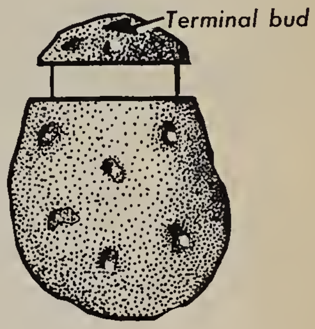


Fig. 23-4-a Prepared potato

Additional research on apical dominance can be done by using *Bryophyllum* leaves. Remove three vigorous young leaves from a potted *Bryophyllum* plant. Prepare them as shown in Fig. 23-4-b, using scissors to cut the transverse section and notch from the leaves. Press the two leaves and the leaf parts of the third leaf into the surface of moist sand in a shallow pan and cover the pan with a sheet of glass. Observe the leaves for the growth of young plants along the leaf margins. (e) Do the young plants appear in the same places on all of the leaves? .... (f) Account for your answer. ....

....

....

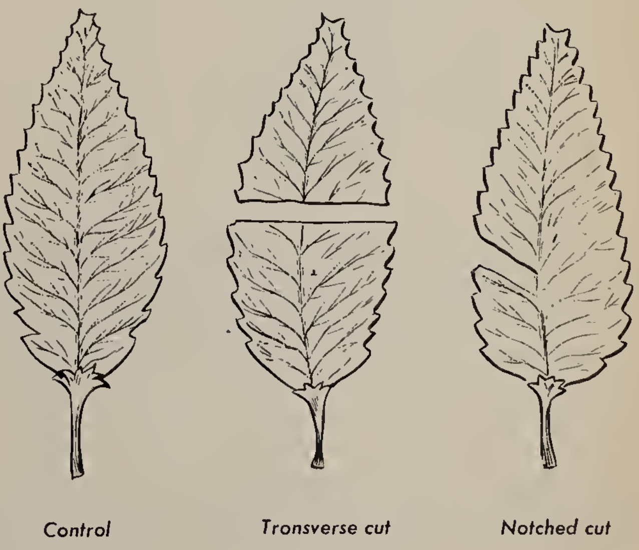


Fig. 23-4-b Prepared *Bryophyllum* leaves

Interpretation. Explain how the experiments in this Part demonstrate apical dominance.

....

....

....

B Part 3 VITAMINS AS PLANT GROWTH STIMULATORS. Vitamin B_1, while not a hormone, stimulates plant growth.

Materials. Vitamin-B_1 solution; 6 seedlings (tomato, zinnia, marigold, bean or other seedlings grown in separate pots); six 3-4 in. flowerpots; potting soil for flowerpots; metric ruler; 6 beakers to hold vitamin-B_1 solution; distilled water.

Procedure and observations. Prepare a vitamin-B_1 solution by dissolving 10 tablets in one liter (1,000 ml) of distilled water. Use six tomato, zinnia, marigold, bean, or other seedlings previously grown in separate pots. Remove the seedlings from the soil and expose the roots. Soak the roots of three of the seedlings in vitamin-B_1 solution and the roots of the other three in plain water for about 30 minutes. Repot all six seedlings. Mark the experimental (vitamin-treated) plants and number them *1* to *3*. Mark the control plants in the same manner. Throughout the experiment, water the experimental plants with vitamin-B_1 solution and the control plants with plain water. Measure the height of all stems at the start of the experiment and at the end of one week, two weeks, and three weeks. Record your results in the table below.

Vitamin-B_1 treated plant	*Stem height at start*	*Height 1 week*	*Height 2 weeks*	*Height 3 weeks*	*Total increase in height*
1					
2					
3					
Untreated plant					
1					
2					
3					

At the close of the experiment, remove all plants from the soil and wash the roots carefully. (a) Are the roots of the experimental plants larger than those of the control plants? .... (b) Are the roots of the experimental plants proportionally larger than other organs of the same plants? ....

Interpretation. What conclusion can you draw concerning the effect of vitamin B_1 on plant growth? ....

....

....

Purpose To demonstrate hydroponics with a mineral solution and determine the effects of mineral deficiencies on the growth of seedlings.

Plants may be grown in coarse gravel by flooding their roots periodically with a nutrient solution containing full mineral requirements. This procedure is referred to as soilless gardening, or *hydroponics*. Hydroponics is often used in research to determine which minerals are required for the growth of certain plants.

B Part 1 GROWING PLANTS BY HYDROPONICS. The solutions used in growing plants by hydroponics must contain all the elements required for growth by the green plant and must contain them in the proper proportions. Some large-scale gardeners use hydroponics to grow roses, tomatoes, snapdragons, lettuce, potatoes, and carrots. Because the essential elements are present, crops grown hydroponically have a higher yield than those grown in soil.

Materials. Stock solutions as outlined under *Procedure and observations;* 6-in. seedlings of tomato, sunflower, or bean; 2 ring stands; 2 funnels 4 in. in diameter; 2 one-hole stoppers to fit in base of flowerpots; two 6-inch glazed flowerpots; 2 pieces of rubber tubing; two 4-inch pieces of glass tubing; 2 ring clamps; balance; 1-liter graduate or volumetric flask; stoppered liter reagent bottle; distilled water; gravel.

Procedure and observations. In preparing a *mineral-deficient solution*, a nonessential solution is substituted for one of the essential mineral solutions. The stock solutions you will make will be used in preparing both full-nutrient and mineral-deficient solutions for experimental use. All stock solutions will be prepared in one-liter quantities, using *distilled water*.

Prepare the following stock solutions:

Magnesium sulfate (Epsom salts)	7.8 gms/liter water
Monocalcium phosphate	4.0 gms/liter water
Potassium nitrate	7.7 gms/liter water
Calcium sulfate	14.2 gms/liter water
Sodium nitrate	6.5 gms/liter water
Potassium chloride	5.7 gms/liter water
Calcium chloride	5.7 gms/liter water
Iron tartrate	4.0 gms/liter water

Weigh the quantities of minerals carefully, using an analytical balance, if available. Mix with distilled water in a thoroughly cleansed liter-graduated cylinder or volumetric flask. Store each stock solution in a stoppered liter reagent bottle and label.

Set up the apparatus as shown in Fig. 23-5-a. The plants may be tomato, sunflower, bean, or any other vigorous plant about six inches in height. The plants must be the same kind and about the same size. Set the plants in the flowerpots and carefully spread the roots as much as possible as the gravel is added. If the pots are not glazed, coat the outer surfaces with melted paraffin to seal the pores. This is best done with a small brush. Insert a glass tube in a one-hole stopper and press the stopper firmly into the hole in the bottom of the flowerpot. A rubber delivery tube extends from the stopper to a funnel mounted in a ring of the ring stand. Adjust the funnel so that the end of the rubber delivery tube is nearly even with the rim of the flowerpot.

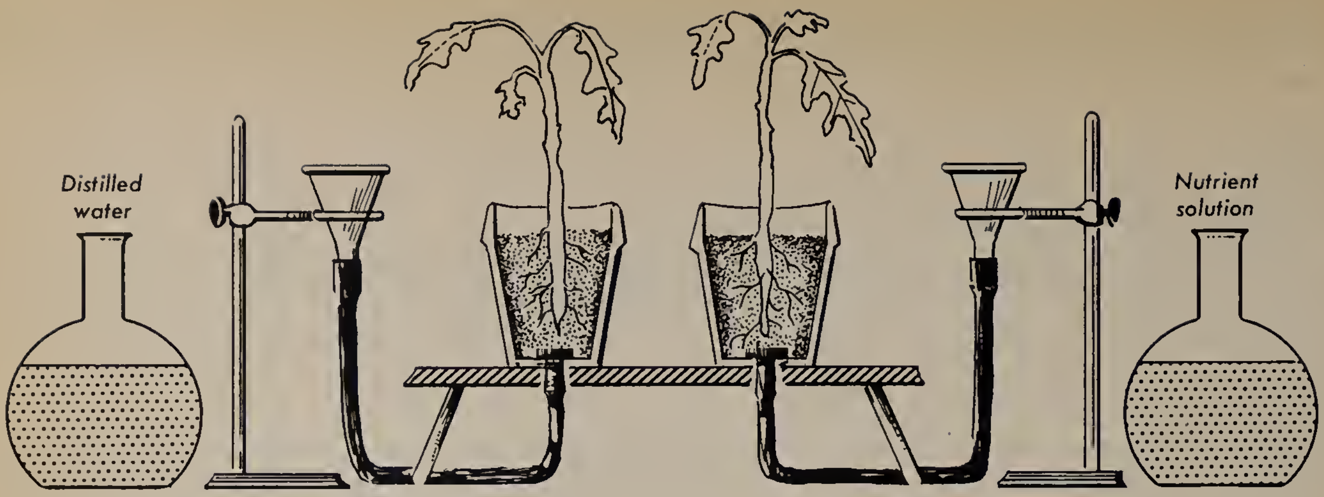


Fig. 23-5-a Apparatus for growing plants with mineral solutions

The apparatus is designed to compare the growth of a plant receiving a full-nutrient solution with a control plant receiving only distilled water. Prepare one liter of full-nutrient (mineral-sufficient) solution from the stock solutions prepared above as follows:

Distilled water	600 ml
Magnesium sulfate solution	100 ml
Potassium nitrate solution	100 ml
Monocalcium phosphate solution	100 ml
Calcium sulfate solution	100 ml

Mix each solution with the water thoroughly as it is added. Store the mineral solution and the distilled water used in the experiment in one-liter flasks.

Pour nutrient solution into the funnel of one of the plants until the level of the solution is an inch below the rim of the flowerpot. Clamp the tube just below the stopper at the base of the flowerpot and allow the solution to remain for 15 minutes. Then remove the tube from the funnel stem, release the clamp, and drain the solution into the flask. Repeat the process with the other plant, using distilled water rather than nutrient solution. Repeat this procedure three times daily (at regularly spaced intervals) for three or four weeks. At the end of each week and at the close of the experiment, record significant data concerning the growth of the two plants in the table below.

Plant	*Week*	*Development of roots*	*Development of stem*	*Development of leaves*
Nutrient solution	1st			
	2nd			
	3rd			
	4th			
Distilled water	1st			
	2nd			
	3rd			
	4th			

Interpretation. How did your experiment demonstrate the vital role of minerals in plant nutrition? ....

....

B Part 2 EFFECT OF MINERAL DEFICIENCY ON THE GROWTH OF SEEDLINGS.

The specific role of various essential minerals in plant growth and development may be demonstrated by growing seedlings in solutions of known mineral deficiency.

Materials. Bean or pea seeds; blotter paper; 2 moist chambers; 4 wide-mouth jars, 5-6 in. in height and 2-3 in. in diameter; wax paper; rubber bands; solutions as given under *Procedure and observations.*

Procedure and observations. These solutions are prepared from the stock solutions with the substitution of one mineral for another.

Prepare one liter of full-nutrient solution as in Part 1.

Prepare three mineral-deficient solutions by substituting for one of the essential minerals, as follows:

Nitrogen-deficient	Substitute 100-ml potassium chloride for 100 ml-potassium nitrate
Phosphorus-deficient	Substitute 100-ml calcium chloride for 100-ml monocalcium phosphate
Potassium-deficient	Substitute 100-ml sodium nitrate for 100-ml potassium nitrate

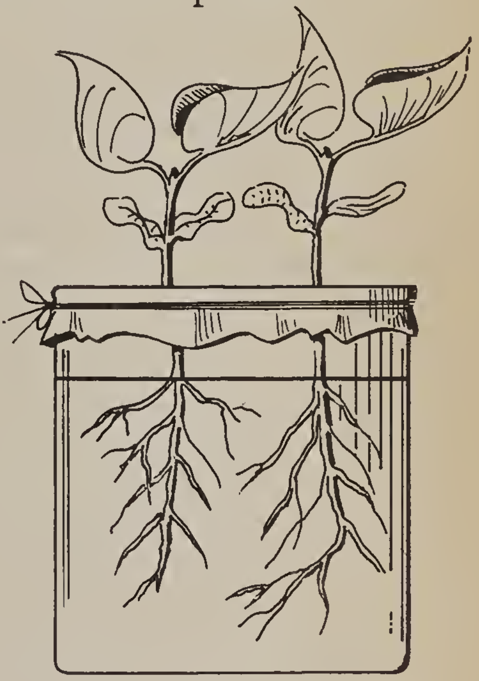


Fig. 23-5-b Mineral solution test jar

Germinate bean or pea seeds between blotters in a moist chamber and grow the seedlings until the roots are three to four inches long. Fill four wide-mouth jars, 5-6 in. in height and 2-3 in. in diameter with the full nutrient and three mineral-deficient solutions. Label each jar. Cut waxed paper covers to overlap the mouths of the jars sufficiently to fasten them with string or a rubber band. Punch three holes equidistant, in the waxed paper, using a pencil point. Carefully insert the roots of two seedlings through two of the holes. The third hole is for adding water as it evaporates. The roots should extend at least two inches into the solution in each jar, as shown in Fig. 23-5-b. Add 2 ml of iron tartrate solution to each jar at the start of the experiment and every two or three days for the duration of the growing period.

Continue the experiment for two to four weeks. Observe the seedlings each day and watch for variations in the rate of growth, size and color of the leaves, patches of discoloration in the leaves, and root growth. At the close of the experiment describe each seedling and any significant changes which occurred in the course of the experiment, using the table provided.

Mineral sufficient	
Nitrogen deficient	
Phosphorus deficient	
Potassium deficient	

Interpretation. Explain how the presence of an element is related to the growth of certain areas of a plant. ....

....

.... Explain why iron tartrate solution was added to the water of the test plants. ....

....

Woody Stems 24-1

Purpose To study the external structure of a woody stem and the structure of a bud; to demonstrate apical dominance in the terminal bud.

You are probably aware that the stems of all trees or shrubs are not identical. An examination of woody stems will reveal that they vary from species to species in external form, size, and internal structure. Woody stems have excellent adaptations for dormancy and are indicators of seasonal changes.

A Part 1 EXTERNAL STRUCTURE OF A WOODY TWIG. Woody stems remain alive and active for more than two years. Their surfaces are covered by cork, and their external structures are very conspicuous. Examination of the external surface of the woody twig reveals leaf arrangement, growth of previous years, and how many branches will form in the next season.

Materials. Woody twig with terminal bud; hand lens.

Procedure and observations. Examine one of your woody twigs closely with a hand lens. The buds are the most conspicuous structures on a dormant stem. Locate the *terminal bud* at the tip of the twig. Notice the bud scales covering the growing point within the bud. Locate the series of rings encircling the twig some distance below the terminal bud. These are *bud-scale scars* left where the scales of the terminal bud of the previous year were attached. The portion of the twig between the terminal bud and the first bud-scale marks the growth of the previous season. The growth of the season before that and other seasons can be observed by measuring between the various bud-scale scars. (a) Count the sections between bud-scale scars and determine the age of your twig.

....

(b) Has growth in length occurred at the same rate each year? ....

(c) Account for your answer. ....

....

....

At intervals along the twig, you will find circular, oval, or shield-shaped *leaf scars,* which mark the point of attachment of leaf petioles from the previous season. Leaf scars are located at the *nodes.* Examine your twig and determine how many leaf scars are located at a node. (d) Classify the leaf arrangement as opposite, alternate, or whorled. ....

External view of woody twig

The space between two nodes is called an *internode.* Examine the twig again and determine the number of nodes produced for each growing season. (e) Is the same number of nodes produced each growing season? .... Examine a leaf scar and notice minute dots called *bundle scars,* which show the location of the conducting vessels that carried water and minerals from the stem into the leaf. (f) Do all the leaf scars have the same number of bundle scars and is the arrangement the same? ....
Look for the *lateral buds* located along the sides of the stem. These are smaller than the terminal bud and are usually different in shape. Lateral buds situated above the leaf scars are *axillary buds.*

Examine the internodes for tiny pores called *lenticels.* These are especially common on younger bark. (g) Describe their form and location. ....

(h) What is their function? ....

....

In the space provided, draw the external view of your woody twig. Label: **terminal bud, axillary (lateral) bud, bud-scale scars, leaf scar. bundle scar, lenticel, node** and **internode.**

A Part 2 **DISSECTION OF A TERMINAL BUD.** A bud is a convex, or cone-shaped, structure formed of meristematic tissue. This embryonic tissue produces stem tissue and small leaves which develop as the bud grows into mature leaves of the plant.

By dissecting the bud and studying the scales individually, the protective nature of the bud scales becomes more apparent. During the dormant period, the meristematic region must be protected from excessive water loss as well as from mechanical injury.

Materials. Terminal bud; dissecting microscope; scalpel or razor blade; forceps.

Procedure and observations. Select one of your twigs with a large terminal bud, if possible. Cut around the base of the bud and remove it from the twig. Make a drawing of the external view of the bud in the space provided.

Now loosen the first (outermost) pair of bud scales, then loosen the second pair and place them above the first pair. Continue until all of the scales are removed. Note the arrangement of the scales as you remove them. (a) Where are the largest scales? .... (b) Why are the largest scales found in this area? ....

....

(c) What is the arrangement of the scales around the bud? ....
(d) Is there any relationship between the arrangement of the bud scales and the arrangement of leaf

External view of terminal bud

Bud scales

scars? ....

....

....

Draw the scales as you arranged them, with the first pair at the bottom, in the space provided. After the scales are removed you should see the *meristematic region* (growing point) of the stem, tiny folded leaves and, in some buds, a flower cluster. Notice the nodes; the internodes may not be visible. Growth of the stem is due to the lengthening of internodes. With your scalpel or razor blade, section the meristematic region lengthwise and examine its parts. Draw the internal structure of this region in the space provided and label: **rudimentary leaves, bud scales, meristematic region.**

Meristematic region, longitudinal section

B Part 3 COMPARISON OF TWIG STRUCTURES. In Parts 1 and 2 you were exploring the individuality of a stem. Now that you are familiar with leaf scars, bundle scars, terminal and lateral buds, you should be able to identify the species of woody twig that you used. If you compare this twig with other twigs, you will observe that there are noticeable differences which can be used in the identification of twigs just as readily as leaves, flowers, and other characteristics.

Materials. Several species of dormant woody twigs; hand lens; identification keys to the trees.

Procedure and observations. Examine the other twigs you collected, determining whether a terminal bud is present or absent, the number of bud scales, number of leaf scars at a node, the number and arrangement of bundle scars, shape of the leaf scar, and other distinctive characteristics. Record all data on the table provided and identify the twig.

Characteristic	*Twig Number*				
	1	2	3	4	5
Terminal bud present or absent					
Number of bud scales					
Number of leaf scars at a node					
Shape of leaf scar					
Number of bundle scars					
Arrangement of bundle scars					
Other distinctive characteristics					
Name of twig					

If you have additional twigs, prepare another table similar to this one and record all the information concerning the twig.

Interpretation. On the basis of your study, explain why a knowledge of winter twig characteristics would be important to a forester. ....

....

C Part 4 APICAL DOMINANCE IN A TERMINAL BUD

Problem. Is there an inhibiting effect of the terminal bud on the growth of other buds?

The growth of growing points in stems is controlled by plant hormones called auxins. Biologists have found that auxin production by the meristematic cells of the terminal bud inhibits the growth of lateral buds, resulting in a prominent stem which continues upward as a central shaft. The plant has a spire-like growth rather than a bushy one. This effect of the terminal bud on other buds is called apical dominance. The florist and gardener take advantage of apical dominance when they cut off the terminal buds of such plants as peonies and chrysanthemums. This allows the lateral buds to develop, resulting in bushy plants and greater production of flowers.

Lightning, insects, and disease are often responsible for the removal of the uppermost stem (leader) in evergreens, so that the first whorl of branches begins to turn upward and one of them becomes the leader. You can study the effect of the terminal bud of the growth of lateral branches by removing the leader on a young tree such as the spruce, pine, or fir.

Suggested materials. Two young trees of the same species; pruning shears.

Suggested procedure. Select two young trees of the same species and of equal age and size. Identify one of the trees as the control and the other as experimental. Cut away the leader of the experimental tree with pruning shears. Do not remove the leader of the control. Watch the growth of the first whorl of branches below the tip of the experimental and control trees for several weeks.

Analysis and conclusions. Your observation of the growth of the lateral branches in the experimental and control trees should result in a better understanding of the influence of terminal buds on the growth of lateral ones.

Stem Tissues

Purpose To examine the tissues of a woody dicot stem, herbaceous dicot stem, and monocot stem.

Trees and shrubs have strong woody stems, while herbaceous plants such as the iris, poppy, bean, and pea have soft stems with little support. A young woody stem is similar in structure to an herbaceous stem except that there is a greater amount of primary xylem, vascular cambium, and primary phloem in the woody stem than in the herbaceous one. As a woody stem grows in diameter, the vascular cambium builds up more secondary tissues, and the outer part of the stem becomes modified.

A Part 1 TISSUES OF A WOODY DICOT STEM. Prepared slides of cross sections of tissues reveal detail that you would not be able to observe macroscopically. For example, you will be able to determine the tissue produced each year by the meristem.

Materials. Microscope; prepared slide of a cross section of a woody stem.

Procedure and observations. Using low power, examine a prepared cross section of a woody stem. Locate the *pith* region which occupies the center of the stem. (a) Describe the pith cells.

....

Study the rays that extend from the pith to the wood. (b) How many cells wide are they?

.... (c) Is the number constant for all rays? ....

(d) What is the function of the pith rays? ....

(e) Where do the rays end? .... Study the slide and find the series of circles, one outside the other. These are the *annual rings,* which mark each season's growth of wood. (f) Do the cells making up an annual ring vary in size? ....

(g) Explain your answer. ....

Examine the *xylem* (wood) from pith to bark by moving the slide. (h) How many layers of wood are present? .... Turn to high power and examine the wood cells. Notice that there are several kinds of cells. The *vessels* are the largest; *tracheids* are smaller and more angular; and *fibers* have very thick walls and small cell cavities. Scattered among these you may find *xylem parenchyma* cells. (i) Judging from their structure, what is the function of the vessels and tracheids? ....

(j) The fibers? .... Locate the *vascular cambium at the* outer edge of the wood. (k) How many layers of cells compose it? ....
The vascular cambium is one of the meristematic regions involved in growth in diameter.

(l) Judging from its location, what two tissues does it produce? ....

(m) Which tissue is produced most frequently? ....

(n) Account for your answer. ....

(o) In relation to the vascular cambium, where is the oldest wood in the stem? ....

.... Locate the *phloem* cells in the inner bark next to the vascular cambium. Phloem consists of several different kinds of cells. Look for the *sieve tubes,* which appear as rows of rather large, elongate living cells that have thin walls and protoplasm but no nucleus. Next to the sieve tubes are narrow *companion cells,* which are also living and lack nuclei. Other thin-walled cells among the sieve tubes are *phloem parenchyma. Phloem fibers* are long, slender cells with thick walls. (p) Judging from their structure, what is the function of the sieve tubes? ....

(q) of the fibers? ....

The outer edge of the inner bark is composed of large, thin-walled cells of the *cortex.* In many young stems the cortex contains chloroplasts and function in photosynthesis. (r) Why is this a temporary function in woody stems? ....

(s) Is there evidence of food storage in the cortex cells? ....

Examine the *cork,* which covers the stem, and the *cork* cambium at the base of the cork. (t) Do the cork cells appear to have been living? .... (u) Explain your answer.

....

(v) Why must the formation of cork be a continuous process? ....

(w) What is the function of the cork cambium? ....

(x) What is the function of the cork? ....

Make a drawing of a wedge-shaped portion of the woody dicot stem and label: **cork cambium, cork, pith ray, cortex, phloem, vascular cambium, xylem, pith, annual ring,** and indicate the **inner bark** and **outer bark.**

Section of a woody stem (L.P.)

A Part 2 TISSUES OF HERBACEOUS DICOT AND MONOCOT STEMS. In this Part you will use prepared slides of cross sections to study the structural differences between a monocot and dicot stem. The conducting and supporting tissue that is so prominent in woody stems is limited to small areas known as fibrovascular bundles in herbaceous stems. The most abundant tissues are soft in texture so that herbaceous plants are limited in the size they can attain.

Materials. Microscope; prepared slide of cross section of an herbaceous dicot (buttercup is good); prepared slide of cross section of an herbaceous monocot stem (corn is good).

Procedure and observations. Shift your slide and examine all parts of the herbaceous dicot stem under low power. The outer edge is covered by a thin epidermis. (a) How many cells thick is it? .... Study the loosely packed cortex cells just inside the epidermis. (b) Do you find any chloroplasts in the cortex cells? .... Within the cortex, the fibrovascular bundles lie in a zone. In some dicot stems they form a continuous ring. In others they are separated by broad rays. (c) How are they arranged in your stem? ....

.... (d) What tissue lies inside the bundle ring? ....

(e) outside? .... Try to find the vascular cambium, which separates the outer portion of the bundle from the xylem tissues within. (f) Why is the growth in diameter of the stem for only one season? ....

Examine a fibrovascular bundle under high power. Make a drawing of the dicot fibrovascular bundle and label: **xylem, vascular cambium, phloem.**

Examine the cross section of the monocot stem. Study all the regions under low power. (g) How does the outer layer of cells differ from those of the epidermis in the dicot stem? .... This is called a rind. (h) What tissue occupies most of the stem? .... Look for the fibrovascular bundles. (i) How are they arranged? ....

.... (j) Is a vascular cambium present? ....

(k) What effect does its presence or absence have on the monocot stem? ....

....

Make a drawing of the monocot fibrovascular bundle and label: **xylem, intercellular space, phloem, mechanical tissue.**

Fibrovascular bundle of herbaceous dicot stem (H.P.)

Fibrovascular bundle of monocot stem (H.P.)

C Part 3 PLANT PROPAGATION BY STEM CUTTINGS

Problem. Can a cut portion of a stem grow new parts to replace lost or damaged ones?

Conduction, support, and storage are the most outstanding functions of a stem. However, reproduction by regeneration is also a function of stems. In nature, stems frequently come in contact with the ground and roots develop. When the stem is severed, the plant becomes fully independent.

You may also be aware that stems of many herbaceous and some woody plants can be rooted by placing them in water or moist sand. These rooted stems are called cuttings.

Suggested materials. Potted geraniums; sandy potting soil; box to serve as cutting bed; plastic to cover cutting bed and plants; scalpel; string.

Suggested procedure. Prepare a cutting bed with three inches of sandy soil. Cut off two branches of the geranium at least five inches long above the bottom pair of leaves. Cut a 2-3 inch piece containing a pair of leaves from the base of one branch. Plant this piece in the cutting bed so that the leaves are just above the surface of the sand. Identify it as specimen 1.

Cut a second section of this branch 2-3 inches long and lacking leaves. Plant this piece in the cutting bed so that one inch of the stem is above the surface of the sand. Identify it as specimen 2.

Plant the other branch so that 2-3 inches of the stem are below the surface of the sand. Identify this branch as specimen 3. Water the plants. Then in order to reduce transpiration, cover the plants and bed with a piece of plastic fastened tightly with a piece of string.

Put the cutting bed in the room where it will receive sufficient light, but where the temperature is 23-27°C. Examine it daily for three weeks and add water as needed. At the end of three weeks, remove the plastic cover and examine the plants to determine whether they are still alive. Carefully examine each specimen for new growth of stem or branches. Remove them from the sand and examine for root growth. Record your observations in drawings and replant the cuttings for future observation.

You may wish to expand your study by using a similar procedure with willow cuttings, coleus cuttings, forsythia cuttings, eyes of the potato, and others.

Analysis and conclusions. From your observations determine whether the presence of leaves made a difference in the rate or amount of growth. You should also be able to determine whether the stem area had any effect on regeneration.

Conduction in Stems 24-3

Purpose To determine the tissues involved in the conduction of water in a stem and the rate of water rise through stem tissues.

When you consider that water may rise as high as 300 feet in a tree, you realize that conduction in a stem is a remarkable process. It is accomplished by a combination of forces including root pressure, capillarity, transpiration pull, and cohesion.

A Part 1 THE PATH OF RISING WATER IN A WOODY STEM. In this Part you will determine which tissues are adapted for conduction of water in a stem.

Materials. Fresh cutting of a maple or elm stem 2 ft or more long, (other woody species may be substituted); 2 branches bearing leaves, 2-3 ft long by ½ in. in diameter at the base, 2 quart jars; 2 ring stands and clamps; scalpel or pen knife; powdered eosin (or red ink); melted paraffin and brush; hand lens.

Procedure and observations. Put the fresh stem cutting in a jar of water immediately to avoid wilting of the leaves. Cut the stem under water six inches above the base to remove the vessels containing trapped air. Add a pinch of eosin or red ink to color the water bright red. Put the jar on the base of a ring stand and clamp the stem in a vertical position. Leave the stem in the solution 30 minutes to several hours.

Remove the stem and split it longitudinally to expose the tissues to the central core of pith.

Examine with a hand lens. (a) Is there stain in the phloem and other bark tissues? ....

(b) in the xylem? .... (c) in the pith? ....

Using the two branches bearing leaves, remove the bark, including the phloem, from both stems for a distance of an inch, as shown in Fig. 24-3-a. Coat the exposed phloem and other bark tissues with melted paraffin on one of the stems, as in Fig. 24-3-a, left. As soon as this stem is prepared, put it in a jar of water and cut the end of the xylem under water, using care not to loosen the paraffin seal. Prepare the second stem as shown in Fig. 24-3-a, right. In this stem, all of the exposed xylem is coated with melted paraffin but the phloem is left open. (Be careful not to plug the phloem while coating the xylem with paraffin.) Put the second stem in a jar of water and clamp both stems in a vertical position on a ring stand. Allow the stems to stand in water for 24 hrs.

(d) Describe the condition of the leaves on the first stem after 24 hrs. ....

.... (e) Explain. ....

....

(f) Describe the condition of the leaves on the second stem.

....

(g) Explain. ....

....

....

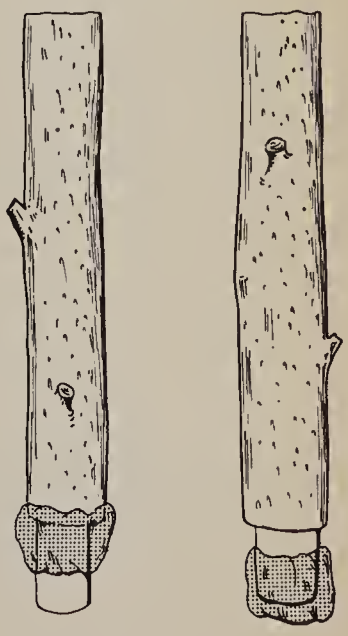


Fig. 24-3-a Tissues involved in water conduction

A Part 2 **THE RATE OF WATER RISE THROUGH A STEM.** The rate of water movement in a stem may be determined by sectioning stems at regular time intervals and examining the vessels through which a colored water solution has been transported.

Materials. Six coleus or periwinkle cuttings 15-20 cm in length; battery jar or 1,000-ml beaker; eosin or red ink, hand lens; scalpel or pen knife; metric ruler.

Procedure and observations. Put all six cuttings in a small battery jar or 1,000-ml beaker. Add a pinch of eosin or red ink to color the water bright red. Spread the cuttings well apart. After 15 minutes, remove one of the cuttings and section the stem longitudinally its entire length. Using a hand lens, examine the tissues and measure the distance the water has risen. Record this distance in millimeters in the table provided. Repeat the procedure at the end of 30 minutes, 45 minutes, and one hour, if possible. Examine the fifth cutting at the end of the school day and the sixth after 24 hours. Record all time intervals and distance of water movement in the table.

Cutting	*Time interval*	*Distance water traveled*
1		
2		
3		
4		
5		
6		

(a) Does the rate of water conduction appear to be uniform in the various cuttings, especially the first hour? .... (b) Did the water solution reach the leaves in any of the cuttings? .... (c) Why was it necessary to use cuttings bearing leaves? ....

....

Tissues of the Leaf 25-1

Purpose To examine the tissues of a leaf in cross section and relate them to their role in photosynthesis and to examine stomates in living epidermis.

The green leaf is the chief site of the photosynthetic process. You have already studied the role of carbon dioxide and light energy in photosynthesis. However, you have not studied the structures of the leaf that are involved in the process. The green leaf is broad, thin, and flat and arranged on a stem so that its surface is exposed to the light. All of the tissues of the leaf are specialized for a particular role in photosynthesis.

A Part 1 TISSUES OF THE LEAF. As you study the leaf tissues in a cross section, you will become familiar with the specialization of leaf structures for absorption of light, conduction of materials, absorption of carbon dioxide, and production of sugar.

Materials. Microscope; prepared slide of a cross section of a leaf (lilac is good).

Procedure and observations. Examine a prepared slide of a cross section of a leaf under low power. When you have found a portion of the section which is especially clear, change to high power. The leaf is composed of three distinct tissues. The first of these is the *epidermis.* Study the upper epidermis. (a) How many cells thick is it? .... The cells of the epidermis are covered by a layer of wax called a *cuticle.* (b) Is a cuticle present? .... (c) What is its function? ....
The *mesophyll* is the largest area of the leaf and is composed of two regions. The first of these is made up of *palisade cells* which lie just below the upper epidermis. Study this area. (d) Describe the cells and their arrangement in relation to the upper epidermis. .... Locate the chloroplasts in the palisade cells. (e) What is the function of the palisade cells? .... (f) Why is the shape of the palisade cells important? .... Study the second region of the mesophyll and find the *spongy layer* of cells below the palisade cells. (g) Which layer is more compact? .... (h) Do you find chloroplasts in the spongy cells? .... (i) If so, are they as numerous as in the palisade cells? .... (j) Account for any difference you find. .... Notice the numerous spaces among the spongy cells. These are *air spaces.* (k) On the basis of their relationship to other tissues in the leaf, what do you think their function is? ....
....

The spongy layer is penetrated by numerous *veins.* If the leaf on your slide was sectioned across a vein, the vein will appear as a group of small, thin-walled and thick-walled, angular cells. In longitudinal section, you can see thick-walled tubes with spirals in the walls. Study your section carefully and see if you can find both cross and longitudinal views of a vein. Study a cross section and find the empty cells with thick walls in the upper part of the section. These are the *xylem* cells. (l) What is the function of xylem cells? .... The thin-walled cells that form a cluster below the xylem cells are the *phloem cells*. (m) What is the function of the phloem cells? .... The smallest veins are composed of only one kind of conducting tissue. Find a small vein in your section near the leaf margin. (n) What kind of cells compose the small vein? ....

Examine the *lower epidermis*. (o) How many layers of cells compose it? .... Look carefully along the lower epidermis to see if you can find tiny pores with small, rounded cells on either side. The pores are the *stomates* and the rounded cells are *guard cells*. (p) What is the function of the stomates? .... (q) What is the relationship between the stomates and the air spaces of the spongy tissue? ....

Make a drawing of a cross section of your leaf in the space provided and label: **cuticle, upper epidermis, palisade cells, spongy cells, vein, lower epidermis, stomates.**

Cross section of a leaf (H.P.)

A Part 2 **STUDY OF THE LOWER EPIDERMIS.** The stomates are easier to find in a surface view of the lower epidermis. The stomate is an intercellular space formed by the splitting of the wall between two specialized epidermal cells, the guard cells. The function of the stomates is to allow carbon dioxide to enter and oxygen to leave the leaf. In this Part you will study the stomate and determine its role in photosynthesis.

Materials. Living leaves (geranium or *Tradescantia*); microscope; slides; cover glasses; dropping pipette; razor blade; paper toweling or filter paper; 10% salt solution.

Procedure and observations. Remove a small area of the lower epidermis from a leaf of geranium or *Tradescantia* by tearing through the blade, twisting slightly as you tear. The epidermis will appear as a thin, transparent skin. Use the razor blade to cut off a small portion of this skin. Mount it in a drop of water on a slide immediately and add a cover glass. (Do not permit the tissue to dry.) Examine with low power. Notice the shape and arrangement of the *epidermal cells* and the number and arrangement of the *stomates.* (a) Do you find chloroplasts in the cells? ....

Stomate and guard cells (H.P.)

Look for the bean-shaped *guard cells* located on either side of the stomates. Turn to high power and study the walls of the guard cells (b) Are the inner and outer walls the same thickness? .... (c) Do guard cells have chloroplasts? ....

Make a drawing of a stomate and guard cells in the space provided. Label: stomate, guard cell.

The stomate is a pore opening into the leaf. Several gases pass through it either into the leaf or out of the leaf. (d) Name three such gases and indicate the direction each would be moving during daylight activity of the leaf. ....

....

.... (e) Which one is important in photosynthesis?

.... The guard cells regulate the size of the stomate by changing shape. (f) Why is this important to the leaf? ....

....

You can demonstrate how the guard cells regulate the opening and closing of the stomate by doing the following experiment. Use the same technique for removing the lower epidermis of a leaf on a plant growing in diffuse light. Mount the leaf and examine it under high power. (g) Are the stomates open? ....

.... Place paper toweling or filter paper at the left edge of the cover glass and remove the water from the leaf. Add the 10% salt solution to the right side and permit it to diffuse under the cover glass. (h) Discuss what happened to the guard cells and stomate. ....

Guard cells and stomate in 10% salt solution (H.P.)

....

Make a drawing of a stomate and guard cells to show these changes. (i) Explain the change that occurred. ....

....

(j) What activity in the leaf might cause the stomates to close? ....

Explain your answer. ....

Transpiration in Plants 25-2

Purpose To demonstrate transpiration in plants and to determine the amount of water lost during transpiration.

During the growing season a continuous stream of water is being conducted up through the roots and stems to the leaves of plants. Some of the water is used for photosynthesis and some for maintaining cell turgor. However, the amount of water actually used by a plant to carry on plant activities is only a fraction of that absorbed by the roots. About 99 percent of the water absorbed by the roots escapes from the leaves and stem as water vapor, in the process called transpiration. Most of the loss of water is through the stomates you studied in Investigation 25-1. The opening and closing of these stomates is regulated by the guard cells. When they are full of water, the stomate is open. When the guard cells lose water, the stomate is open. When the guard cells lose water, they change shape, the stomates close, thus reducing water loss.

A Part 1 TRANSPIRATION IN PLANTS. The loss of water by transpiration can be demonstrated by using a vigorously growing potted geranium plant.

Materials. Vigorously growing potted geranium plant; 2 bell jars; cobalt paper; piece of aluminum foil or rubber sheet to wrap the flowerpot; string; rubber band.

Procedure and observations. Water a potted geranium and allow it to stand until all of the water has entered the soil and until none is dripping from the hole in the bottom of the pot. Set the plant in the center of a sheet of aluminum foil or rubber sheet. Draw the foil or rubber sheet over the pot and wrap it around the stem of the plant (Fig. 25-2-a). Fasten the foil or rubber sheet tightly around the stem with string or rubber band. Set the plant in a sunny place and cover it with a large bell jar. Set an empty bell jar beside it as a control. Put a piece of cobalt-treated filter paper under each of the bell jars. (Cobalt paper is an indicator of moisture.) (a) What color is the paper at the beginning of the experiment? ....

Observe the paper at the end of the class period. (b) What color is the paper under the jar containing the geranium plant? .... (c) the empty jar? .... (d) Account for any difference in color of the two pieces of cobalt paper. ....

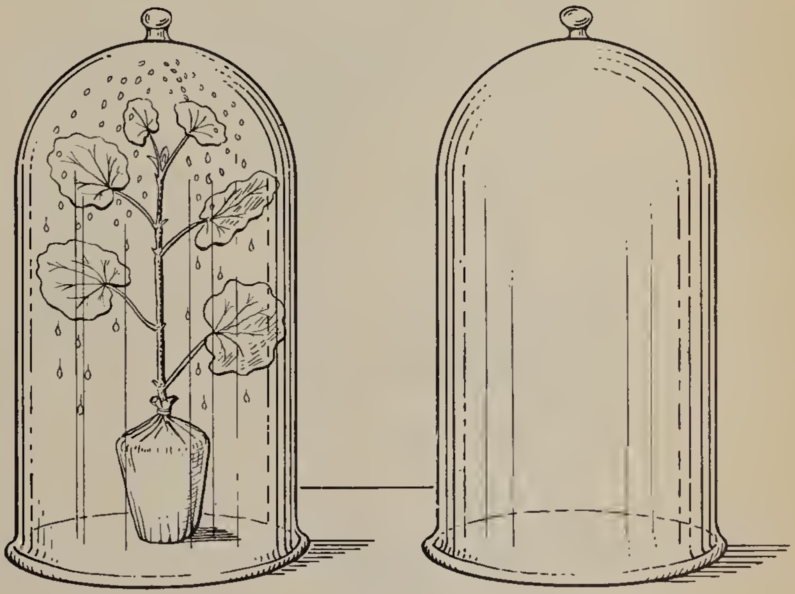


Fig. 25-2-a Transpiration experiment

Study the walls of the two bell jars at the beginning of the period on the following day. (e) Describe the walls of the bell jar containing the geranium plant. ....

(f) the empty bell jar.

(g) Account for any differences you found.

(h) Why was the pot sealed during this demonstration?

(i) Why was a control jar necessary?

(j) From your observation of this demonstration, write a summary definition of transpiration.

B Part 2 DETERMINATION OF THE AMOUNT OF WATER LOST DURING TRANSPIRATION. The amount of water lost from a plant by transpiration can be determined by using a potted geranium and weighing it at intervals.

Materials. Potted geranium, balance; piece of aluminum foil or rubber sheet to wrap flowerpot; string; rubber bands.

Procedure and observations. To avoid loss of water from the soil, wrap the pot in a sheet of aluminum foil or rubber sheet. Draw the foil or rubber sheet over the pot and wrap it around the stem of the plant (see Fig. 25-2-a). Fasten the foil or rubber sheet tightly around the stem with string or rubber band. Weigh the plant, and record the initial weight in the table provided. Put the plant in a well-lighted area of the room. Continue to weigh the plant daily for a period of a week, recording the daily weight in the table provided. Determine the total loss of weight in the plant for the week and record in the table. (a) What percent of the initial weight was lost through transpiration?

(b) Why didn't the leaves of the geranium wilt?

(c) List as many factors as you can think of that affect the loss of water from a plant.

Initial weight	
Weight 2nd day	
Weight 3rd day	
Weight 4th day	
Weight 5th day	
Total loss	

Interpretation. On the basis of what you have learned about transpiration, account for the fact that leaves often wilt on a hot summer day, but return to a normal condition at night.

The Flower—Specialized Reproductive Structure 26-1

Purpose To examine the parts of a flower.

The biologist defines the flower as the reproductive structure of the angiosperms. Within the flower, fertilization occurs and the fertilized ovary develops seeds—the structures by which angiosperms maintain the species.

A Part 1 STRUCTURE OF A FLOWER. A *complete* flower is one that contains all four main parts: the calyx, corolla, stamens, and pistil. As you study the various flowers, you will learn that many of them are *incomplete,* lacking one or more of the main parts. The stamens and pistil are essential parts in reproduction, while the calyx and corolla are only accessory parts. Thus, where the stamens and pistil are both present in the same flower, even though the sepals and petals are missing, the flower is said to be *perfect.* If either the stamens or pistil is missing, the flower is *imperfect.* A flower containing only stamens is said to be *staminate,* while one having only pistils is a *pistillate* flower.

Materials. Large flower (tulip, gladiolus, lily, daffodil, sweet pea); hand lens; microscope; slide; cover glass; scalpel.

Procedure and observations. Section 1 *Types of flowers.* (a) Describe the flowers in Fig. 26-1-a, in the blanks provided on the next page, by using all the following terms that are applicable: *complete, incomplete, perfect, imperfect, staminate, pistillate.*

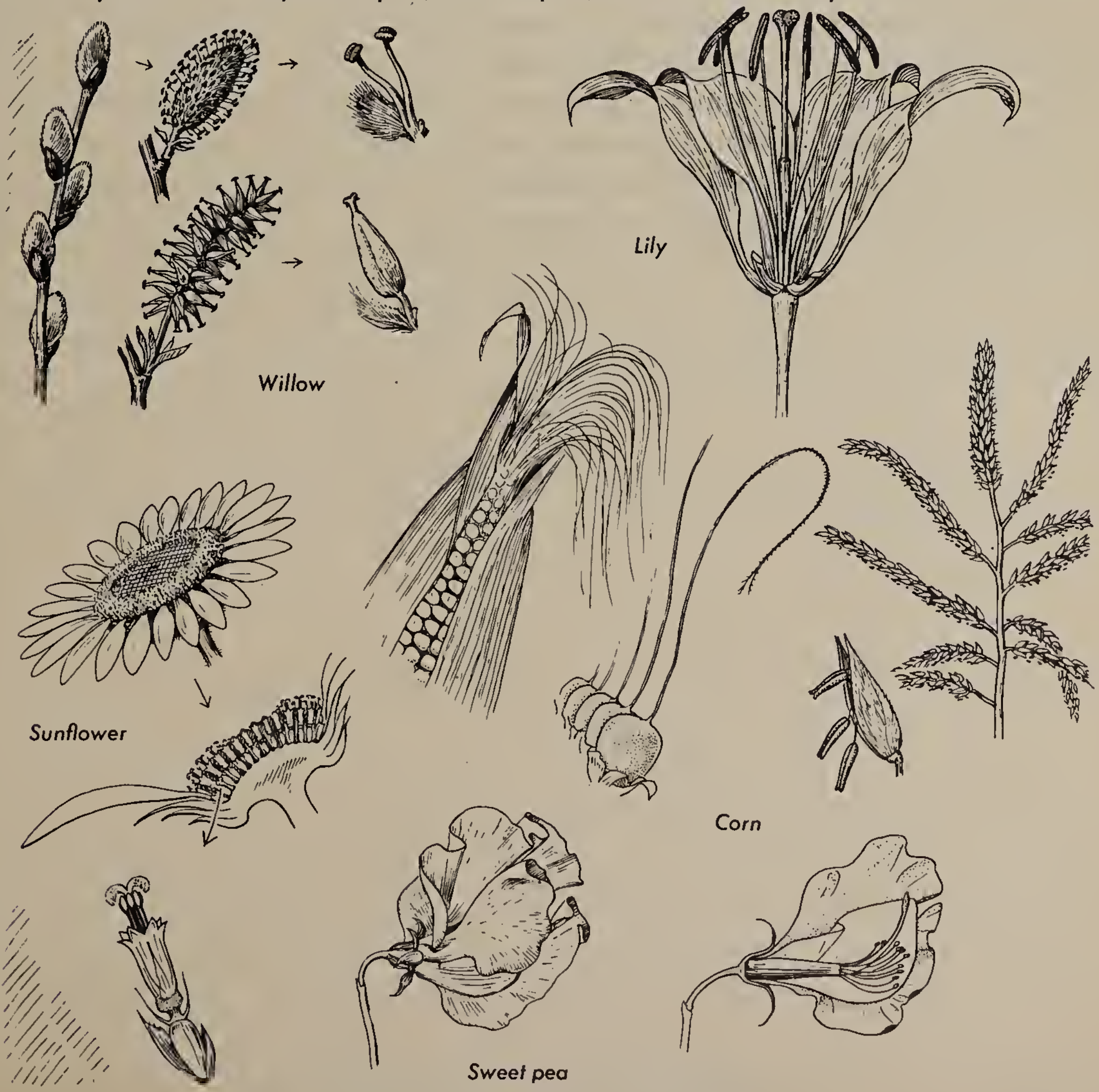


Fig. 26-1-a Types of flowers

Characteristics

1.
2.
3.
4.
5.

Study the flower specimen you have. (b) How many sepals are in the calyx?

(c) How many petals are in the corolla? (d) How many pistils and stamens are there? (e) How are the stamens arranged for your specimen?

Remove one of the petals, cut a small section from it and mount in a drop of water on a microscope slide. Examine the section under low and high power. (f) Can you locate the chromoplasts? (g) What is their function?

Section 2 *Essential parts.* Using your forceps, remove one of the stamens. Lay it on a sheet of white paper and examine it with your hand lens. Note the slender stalk, or *filament,* and the enlarged knob on the end, the *anther.* Draw the stamen in the space provided and label its parts. With a scalpel or razor blade, section the anther lengthwise. This will reveal the *pollen chambers.* Draw the internal structure of the anther. The structure in the center of the flower is the *pistil.* Its enlarged tip is the *stigma;* the slender part in the middle is the *style;* the swollen part at the base is the *ovary.* Study the pistil with your hand lens and make a drawing. Label its parts. Section the ovary lengthwise and count the number of ovules present. Draw the sectioned ovary in the space provided. (h) What is the relationship between the number of ovules in the chambers and the number of petals and stamens in your flower?

(i) Is your flower a monocot or dicot?

Stamen (enlarged)	Anther (sectioned)	Pistil (enlarged)	Ovary (sectioned)

Structure and Germination of Pollen Grains 26-2

Purpose To examine pollen grains and determine the variations in pollens of different species; to observe germination of pollen grains and formation of tubes; to make a comparison of the pollen produced by species within a particular genus; and to study generic similarities in the structure of pollen grains.

At the time pollen is shed from the mature anther of a stamen, the outer wall of the microspore has thickened and become a protective covering, or *exine.* This outer wall may develop spines, ridges, plates, pits, or other markings which are characteristic of pollen grains of a species. Thus, pollen grains are just as distinctive as are leaves, flowers, fruits, or seeds, and may be used as a basis for plant identification.

A Part 1 EXAMINATION OF A VARIETY OF FLOWER POLLENS. The outer margins of the pollen grains are very important in distinguishing the pollen produced by various flowers. In this Part you will become familiar with some of the characteristics of pollens.

Materials. Collection of anthers from a variety of flowers; microscope; slides; cover glasses; dissecting needle or small brush; Bunsen burner; crystal violet or methylene blue.

Procedure and observations. Collect stamens from a variety of flowers and place them in separate small envelopes. Your collection should include a variety of plant genera and families represented in such common flowers as the tulip or lily, daffodil and narcissus, rose, gladiolus, snapdragon, dandelion, marigold, zinnia, various species of grasses and, if available, pollen from several trees. Conifers, such as the pine, spruce, or fir are interesting if staminate cones are available. Mount each kind of pollen in a drop of water on a slide, using a dissecting needle or a small brush to transfer pollen from the anther to the slide. Pollen will probably be clinging to the sides of the envelope and can be transferred easily. Add a drop of crystal violet or methylene blue stain to the drop of water and allow the pollen grains to stain for three to five minutes. If the pollen grains do not absorb stain readily, *warm* the slide over a Bunsen flame, using care not to heat the slide. Examine with low power of the microscope to be sure that the pollen grains are stained, then add a cover glass. Study the pollen grains under low power or high power (depending on their size).

In the space below, draw a variety of pollen grains, using care to show all distinctive characteristics of the outer wall accurately. Name each pollen grain and indicate the magnification used.

Pollen grains

B Part 2 GERMINATING POLLEN GRAINS. When pollen grains are mature and shed from a ripened anther, they are ready to germinate and form pollen tubes. Normally this occurs on the stigma of the flower pistil. However, pollen grains will form pollen tubes under laboratory conditions if they are put in a sugar solution. Pollens from different species of plants require different concentrations for germination. However, most pollens germinate well in a 10% sucrose (cane sugar) solution. The best guide to the sugar concentration required for germination is the stickiness of the stigma of the flower from which the pollen is obtained.

Materials. Collection of pollen grains; microscope; slides; slide rings for hanging drops; cover glasses; dissecting needle or small brush; petroleum jelly; dropping pipette; 10% sucrose solution; distilled water.

Procedure and observations. Prepare a 10% sucrose solution by adding 1 gram of sugar to enough distilled water to make 10 ml of solution (or 10 grams of sugar and water to make 100 ml of solution). Mix the sugar and the water thoroughly in a graduated cylinder, then pour the solution into a beaker for use. Put a drop of sugar solution in the center of a cover glass and transfer pollen grains to the drop with a dissecting needle or small brush. Invert the cover glass and place it over the depression of a culture slide or on a hanging-drop ring on a standard slide. The slide depression must be deep enough to prevent the drop from touching. Seal the cover glass to the slide or ring with petroleum jelly to prevent drying out. Examine with low power of the microscope. Pollen tubes should emerge within 20 minutes and may continue to grow for several hours. Certain pollens may require one or two days to produce pollen tubes.

In the space provided, draw a pollen grain at the start of the experiment and several stages in the growth of the pollen tube. Notice especially the nuclei in the pollen tube as it grows. Indicate the time interval in each successive stage you show and label all significant structures.

Germination of a pollen grain

Interpretation. From your observations of germinating pollen, determine in what way the sugar solution produced a condition similar to that which causes natural germination of pollen grains. ....

....

B Part 3 VARIATION IN THE GERMINATION OF POLLEN GRAINS. Different kinds of pollen vary in the concentration of sugar that induces germination and in the rate at which the pollen tube grows. For example, composites require concentrations between 30 to 35% sucrose solution. Growth of the pollen grains should begin within three hours at room temperature (23°C or 70°F).

Materials. Collection of pollens; microscope; culture (concave) slides; cover glasses; micrometer or metric ruler; sucrose solutions (5%, 10%, 15%, 20%, 25%, and 30%).

Procedure and observations. Prepare a series of sucrose solutions as follows: 5%, 10%, 15%, 20%, 25%, and 30%. Using the same kind of pollen, make hanging drops of each sucrose solution, as outlined in Part 2. Compare the rate of growth of pollen tubes in each solution at regular intervals. Measure the length of pollen tubes at each interval with a micrometer eyepiece or a millimeter scale placed on the microscope stage under the slide. Compile your data in the table provided, using the spaces to record the length of the pollen tube at each interval of measurement.

	Growth of Pollen Tube in Sucrose Solutions					
	5%	10%	15%	20%	25%	30%
Time Interval						

Use a 10% glucose solution to make hanging drops of pollen from six or more plant species. Measure the length of the pollen tube at various time intervals and record in the table provided. At the top of each column, indicate the species of pollen. Record the time of measurement in the left column.

Growth of Various Pollen Tubes in a Ten Percent Sucrose Solution							
Time of Measurement							

Still another experiment may be conducted in which you use one species of pollen and 10% solutions of glucose, maltose, or lactose. Interesting results are obtained from the use of honey. Prepare a table for summarizing your results.

Interpretation. Using the data in your tables, explain how you have demonstrated that pollens of different plant species vary in their requirements for germination. How would this reduce the chance that pollen grains might begin to germinate on the stigma of a flower of a foreign species?

C Part 4 GENERIC SIMILARITIES IN THE MORPHOLOGY OF POLLEN GRAINS

Problem. Do the pollen grains of various members of a genus resemble one another in shape, size, and surface (exine)?

You have studied pollen grains from various types of plants and found that they differ from one another in many respects. Would you expect the members of a genus to produce pollens that are similar to one another and distinct from those of other genera? You can demonstrate the similarities or differences in pollen grains of members of a species by examining them under the microscope.

Suggested materials. Collection of pollen from species of plants within the same genus; microscope; culture (concave) slide; cover glasses; dissecting needle or small brush; Bunsen burner; crystal violet or methylene blue.

Suggested procedure. Collect the stamens from several species of plants of the same genus, e.g., pines, spruces, maples, composites, or lilies. Prepare mounts of the pollen grains as directed in Part 1 of this Investigation. Examine the pollen grains under low and high power of the microscope. Study the surface (exine) and shape of the pollen grains. Using the method in Investigation 1-2, measure the pollen grains.

Analysis and conclusions. Summarize your results in a table and in the form of drawings. Your study of pollen grains should further illustrate the genetic relationship of members of a genus.

The Fruit—A Matured Ovary

Purpose To study the structure and classification of typical fleshy and dry fruits.

The fruit of a plant is the ripened ovary and its contents and any adjacent parts that may be connected to it. Fruits arise from floral organs and are found only among flowering plants. The fruits of flowering plants are classified as dry or fleshy according to their structure.

A Part 1 FLESHY FRUITS. The apple is an example of the kind of fleshy fruit called a *pome.* Its outer, edible layer develops from the calyx and receptacle of the flower. The peach, plum, and cherry are fleshy fruits called *drupes,* or stone fruits. The various citrus fruits and berries are also types of fleshy fruits.

Materials. Apple, peach, cherry, or plum; knife or razor blade.

Procedure and observations. Examine the stem of an apple. The stem itself is the stalk, or *peduncle,* by which the flower was attached. At the other end are the remains of the *sepals,* and a ring of dried *stamens.* Section the apple lengthwise. The thin skin is an *epidermis.* Next comes the fleshy *cortex* formed from the *calyx* and *receptacle.* Just outside of the papery core is a line formed by a ring of fibrovascular *bundles.* The papery core is the *ovary wall.* Within the ovary are the *seeds,* attached to a central *placenta* which runs through the *ovary.* (a) Can you name another pome fruit? .... (b) From what part of the flower is the fleshy part of this kind of fruit formed? ....

In the space at the left below, draw a view of the cut apple. Label: **remains of sepals, fleshy receptacle, ovary wall, placenta, seeds.**

Pome fruit **Stone fruit**

Section the peach, plum, or cherry of these lengthwise. Remove the pit and crack it. Lay the half containing the seed back in the fruit. The outer covering of the drupe is a thin *epidermis* (skin). The *ovary wall* develops in two layers, an outer *fleshy layer* and a hard *thin layer* which forms the pit. In the pit is the *seed.* In some drupes the fleshy layer is edible; in others, the seed. (c) Which part is eaten in the olive? ....

(d) in the walnut? .... (e) in the almond? ....

(f) in the plum? .... (g) From what part of the flower does the

fleshy part of stone fruits develop? ....
In the space at the right on page 229, draw a view of a cut stone fruit and label: **epidermis, fleshy layer, stony layer** (*pit*), **seed.**

A Part 2 **DRY FRUITS.** The pod fruits such as the bean and pea are classified as dry fruits. All the various nuts and grains are dry fruits, as are the winged fruits produced by the maple, ash, and elm.

Materials. Bean or pea pods; parachutes of dandelion or goatsbeard; razor blade or knife.

Procedure and observations. (a) Why is a legume fruit (pod) of the bean and pea called dehiscent? ....
Examine a bean or pea pod and find the stem, or *peduncle.* The *calyx* is very noticeable at the stem end of the pod. The pod is the enlarged *ovary.* It ends in a pointed structure which is the remains of the *style.* Draw and label in the space provided: **peduncle, ovary, calyx,** and **remains of the style.** Now, open the pod along the lower side. The *seeds* are arranged in rows attached to a *placenta* which runs along the top of the pod. Draw the opened pod and label: **seed, placenta.**

Examine a dandelion or goatsbeard fruit. The seed is in the hard case at the lower end of the fruit. The fluffy parachute is known as the *pappus.* This type of fruit is called an *achene.*

(b) Why is the achene an example of an indehiscent fruit? ....
.... Draw and label in the space provided: **seed, pappus.**

Dehiscent dry fruit

Indehiscent dry fruit

The fruits we eat are formed from flowers which have been pollinated and fertilized. After this takes place, the showy flower parts fall off and certain structures begin to develop into the fruit. In the following table indicate which structures of the flower develop into the parts we eat.

Name of fruit	*Structures of flower from which it developed*
1. Pear	
2. Peach	
3. Grape	
4. Strawberry	
5. Blackberry	

The Seed—A Matured Ovule

Purpose To study the structure of a dicot and monocot seed.

A seed is a matured ovule and the final product of angiosperm reproduction. It is composed of an embryo plant, stored food, and one or more seed coats.

A **Part 1 STRUCTURE OF SEEDS.** The lima bean is an example of a dicot seed, and the corn kernel of a monocot seed. Actually, the corn kernel is a fruit, but the seed makes up all of it except a thin outer covering.

Materials. Lima bean; corn kernel; glass dish; knife or razor blade; hand lens; iodine solution.

Procedure and observations. Soak a lima bean and a corn grain overnight. Study the structure of the lima bean first. Observe the thin outer seed coat, the *testa*. Examine the concave side of the bean and locate the *hilum*, which marks the point where the seed was attached to the pod. With your hand lens find the tiny pore, or *micropyle*, directly above the hilum. (a) What function does the micropyle serve? ....
Lay the bean on its side and draw an external view in the space below. Label: **testa, hilum, micropyle.**

Remove the testa carefully and expose the *cotyledons.* (b) Describe the cotyledons.

....

(c) What is their function? ....
Locate the *hypocotyl*, which projects through the cotyledons. Separate the cotyledons so that epicotyl and hypocotyl remain attached to one of them. Examine the *epicotyl leaves* with a hand lens. At the base of the leaves is the *scar* where the cotyledons were separated. Add a drop of iodine to the testa, cotyledon, epicotyl, and hypocotyl. (d) Which structure contains the greatest amount of starch? .... Draw the bean with one cotyledon removed in the space below and label: **cotyledon, epicotyl, cotyledon scar, hypocotyl.**

Bean (external)

Bean (internal)

Examine both sides of the corn grain. On one side you should find a prominent dent marking the location of the cotyledon and embryo plant. At the base of the grain is a pointed projection, the *point of attachment* to the cob. Another projection, at the top of the grain, is called the *silk scar.* At the point, the silk, or style, was attached to the grain during the flowering stage. Examine all parts of the grain with your hand lens and make a drawing in the space provided of the enlarged grain showing all parts described. Label: **silk scar, point of attachment.**

Position the grain with the silk scar, cotyledon, and embryo mark facing up. Using a sharp knife or razor blade, cut lengthwise completely through the grain. Turn the grain with the sectioned portion facing up and examine with a hand lens. The outer covering is the *ovary wall.* The lower portion contains the *embryo* and *cotyledon.* The upper part of the embryo is the *epicotyl sheath.* Directly below it is the *hypocotyl.* The *cotyledon* is attached to the epicotyl and the hypocotyl. The bulk of the seed is a tissue called the *endosperm* and contains stored food. Add a drop of iodine to the endosperm and observe. (e) What color appears?

.... (f) This color is a test for what food? ....

(g) In what form is food stored here? ....

Draw the sectioned grain in the space provided and label: **ovary wall, endosperm, cotyledon, epicotyl, hypocotyl.**

Corn (external)

Corn (internal)

Seed Germination 26-5

Purpose To study the germination of seeds and to test seed viability.

Seed germination begins with the growth of the embryo and is completed when the radicle is formed outside the seed. Germination is a critical period in the life of a plant; during this time the greatest number of plants are lost.

A Part 1 STEPS IN GERMINATION OF A DICOT AND MONOCOT SEED. In this Part you will study germination in a monocot seed and in a dicot seed and the differences between the two.

Materials. Bean seeds; corn seeds; glass tumbler; paper towel; blotter or filter paper; sawdust.

Procedure and observations. Prepare a germination glass by cutting a strip of paper towel or blotter to line a glass tumbler. Fill the inside of the glass with sawdust. Put several bean and corn seeds in a row around the glass in the space between the liner and the outside of the glass. Moisten the sawdust so that it is damp, but avoid excess water. Put the germination glass in a warm place and keep the sawdust moist. Allow the seeds to germinate until the young seedling plants are well formed. Observe the development of each type of seed daily, and sketch in the spaces below the main steps of germination of each seed as they occur.

Steps in the germination of a bean seed

Steps in the germination of a corn seed

(a) List the differences in germination of the corn and bean seed. ....

....

....

....

....

B Part 2 CONDITIONS NECESSARY FOR PROPER GERMINATION OF SEEDS. In this Part you will study the environmental requirements for germination of corn and bean seeds.

Materials. Bean seeds; corn seeds; refrigerator; 4 glass tumblers; glass-marking pencil or gummed labels; sawdust; paper towel, blotter or filter paper.

Procedure and observations. Prepare four germination glasses as before, and number the glasses with a label. In glass 1 do not moisten the sawdust at all during the course of the experiment. In glass 2 add an excess of water constantly. In glass 3 add a normal amount of moisture but keep the glass in a very cool place. (A refrigerator, if available, is excellent for this part.) In glass 4, add water normally but keep the glass at room temperature. Allow these glasses to stand for a week and observe any daily changes that occur. Record your results in the following table, giving briefly the degree of development of the roots and stem in each group of seeds.

Germination glass		*Development of root*	*Development of stem*	*Condition for germination absent*
1	Bean			
	Corn			
2	Bean			
	Corn			
3	Bean			
	Corn			
4	Bean			
	Corn			

Interpretation. On the basis of your observations, discuss the conditions necessary for proper germination of seeds. ....

....

B Part 3 SEED VIABILITY TESTING. The ability of seeds to germinate is called *viability*. The percentage of viability of a given type of seed is particularly important to the commercial seed grower.

Materials. Two pieces of muslin (or burlap); 100 seeds of the same species.

Procedure and observations. Wet a piece of muslin or burlap and lay it out. Select 100 seeds of the same kind of plant and lay them out in rows, spaced well apart on the cloth. Wet the other piece of cloth and lay it over the seeds carefully. Roll the two pieces loosely. This device is called a "rag-doll tester." Keep the tester moist for several days or a week, checking regularly to see if the seeds have sprouted. When the seeds have germinated, unroll the cloths and count the number of seeds which have sprouted. Record this number as the percentage of viability.

Interpretation. Cite as many reasons as you can to explain the inability of some seeds to germinate.

Porifera—The Sponges 27-1

Purpose To observe the structure of some common types of sponges and to study regeneration in the sponge.

The sponges are the first group of organisms considered animals in the more recent classifications. They are of special interest because they reveal a unique plan of body structure which, in one sense, might be considered a large colony of animal cells and, in another, a multicellular organism.

In the study of protozoans, we found cell structures specialized to a high degree, thus permitting a single cell to live a solitary existence with great efficiency. In the sponge, we find cellular "teamwork" in a functional division of labor, where each cell benefits from association with other cells. There are specialized cells which form an outer covering layer and the pores through which water enters the sponge body. Other specialized cells provide the beating cilia which create the water currents, while still others digest food and pass nourishment to dependent neighboring cells, form the supporting skeleton, and function in reproduction. However, with all of this cell specialization, there is little cell coordination.

A Part 1 GRANTIA (SCYPHA), A SIMPLE SPONGE. The sponge you will examine in this Part is a simple marine sponge of the sycon type. While it is commonly referred to as *Grantia,* a more correct name is *Scypha.* While you will not see the cells composing *Grantia,* you can observe many of its gross structures and compare it with diagrams of sponge types.

Materials. Specimen of *Grantia* (*Scypha*); hand lens; scissors; probe or dissecting needle; watch glass.

Procedure and observations. Examine the outer surface of the animal with the aid of a hand lens. (a) Describe its appearance. ....

.... (b) From its external appearance, identify its type by comparing it with diagrams A, B, or C. .... Notice the openings in the surface. Insert a dissecting needle in one of the openings. (c) Does it pass through the body wall? ....

(d) On the basis of your answer to *c,* explain the function of the opening. ....

.... Notice the opening at the tip of the sponge. (e) What is the function of this opening?

....

....

Insert the point of the scissors into the *osculum* of your specimen and carefully cut the animal lengthwise. With the hand lens, locate the two layers of cells and the hollow central cavity of the sponge. Diagrams D, E, and F show that the *canals* are provided with *flagella.* (f) What is the function of the flagella? .... Compare your section of sponge

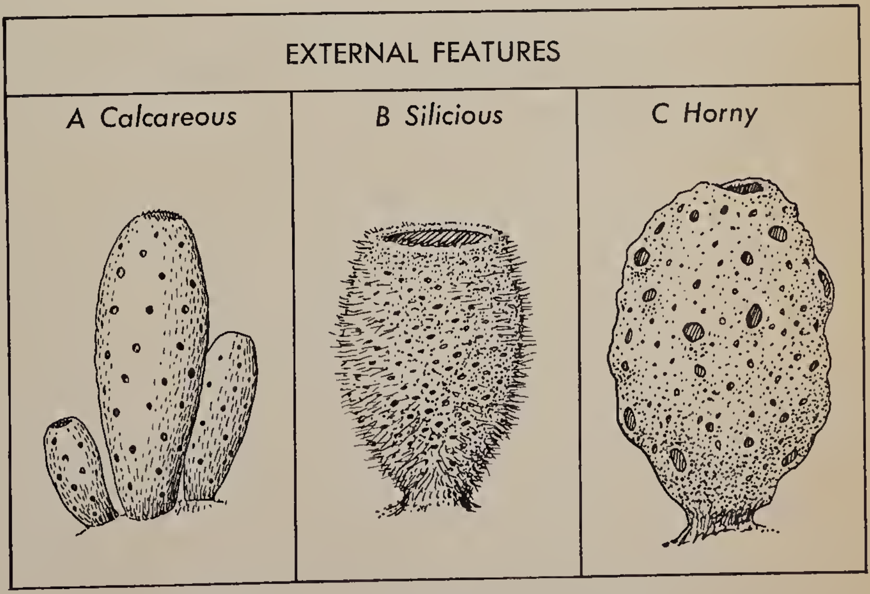


Fig. 27-1-a Three types of sponges

with diagrams D, E, and F. (g) Which type does your sponge resemble? .... As much as 40 gallons of water may pass through the sponge in a single day. (h) How does this large water requirement indicate that sponge cells are very active? ....

....

....

On diagram A, B, or C, label: **spicules, pores, osculum.** On diagram D, E, or F, label: **canals, central cavity, flagella.**

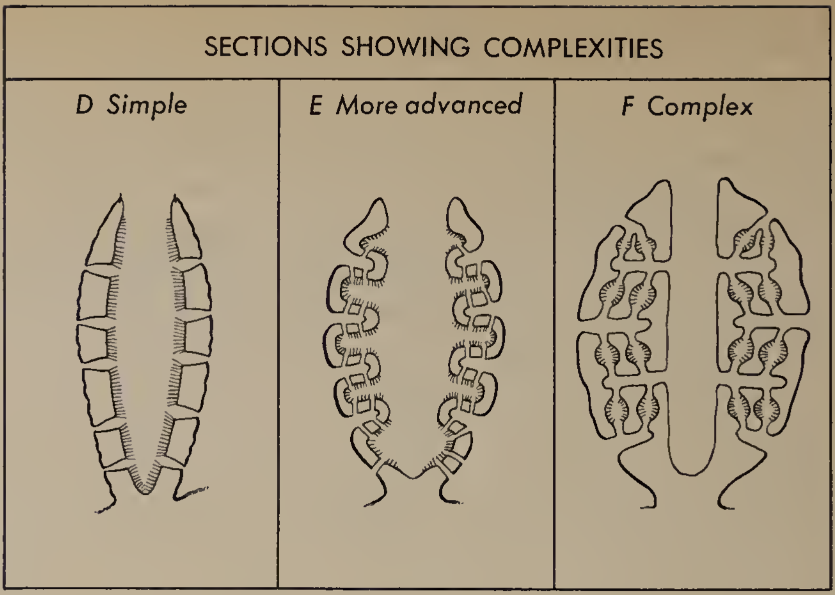


Fig. 27-1-b Three types of sponges

C Part 2 REGENERATION IN SPONGES

Problem. Can a sponge be formed from dissociated sponge cells?

Many of the primitive animals have some ability to replace injured or lost parts through the process of regeneration. Since the sponge has little specialization, you might expect that individual clumps of cells could give rise to a new sponge. You can observe the behavior of sponge cells by forcing sponge tissue through fine mesh cloth and allowing them to orient themselves in a culture dish of sea water.

Suggested materials. Living specimen of sponge (*Microciona*); fine mesh cloth such as that used for sifting flour in flour mills; culture dish; scissors; hand lens or stereoscopic microscope; ice; clean sea water.

Suggested procedure. Cut small pieces of the living sponge and force them through fine mesh cloth into a culture dish containing sea water. Keep the water cool by putting ice around it. Use your hand lens to observe the movement of the cells. Notice what happens when they come in contact with one another. Allow the masses of cells to stand for several hours; then use the hand lens to examine them again.

Analysis and conclusions. You should be able to conclude from your observations the extent to which sponges are capable of regeneration. Relate their regeneration to the degree of specialization in sponges.

Purpose To study the structure and behavior of the living hydra, the structure of *Aurelia,* and regeneration in the hydra.

The coelenterate, with its beginnings of a digestive cavity, nerve cells, and some organization at the tissue level, is considered somewhat more advanced than the sponge. Many of the coelenterates have radial symmetry. They have a body wall consisting of two layers and a gastrovascular cavity that connects to the outside by a mouth. There are two body forms in this phylum; the polyp, which is tubular and the medusa, which is bell-shaped. The polyp is sessile and has the mouth at the anterior end. The medusa is free swimming and has the mouth opening on the ventral side. Many coelenterates have an alternation of the polyp stage with the medusa stage.

A Part 1 STUDY OF A LIVING HYDRA. The hydra is a simple coelenterate found in fresh-water ponds and streams. It has no alternation of stages, occurring only in the polyp stage. This tiny animal (one-quarter to one-half inch in length) attaches itself to plants or debris in the water.

Materials. Hydra culture; culture of *Daphnia;* microscope; culture (concave) slides; dropping pipette; probe; two flashlights; piece of blue cellophane paper; Syracuse watch glass; acetic acid; carmine red.

Procedure and observations. With a pipette, transfer a hydra to a concave slide. Study the organism under low power. (You may find that you can see the animal better in a dark field.) Locate the *basal disk,* by which the animal attaches itself to plants or debris in the water. (a) Does the animal appear to have any supporting framework? .... Notice the tentacles, which are characteristic coelenterate structures. (b) How many tentacles does your specimen have? .... Locate the *mouth,* which is a small slit at the anterior end between the tentacles. The hydra is composed of two layers of cells, an outer one called the *ectoderm* and an inner one called the *endoderm.* Using high power, locate these cell layers and the *gastrovascular cavity* within the entire animal. With your fine adjustment, focus on the body and locate the *nerve net.* This lies in the jellylike layer between the ectoderm and endoderm, called the *mesoglea.* Focus on the tentacles and locate the *nematocysts* (stinging cells), which are structures unique to the coelenterates. Draw a hydra in the space provided and label: **tentacles, mouth, gastrovascular cavity, ectoderm, endoderm, nerve net, nematocyst.**

Structure of the hydra

Study the movements of the hydra under low power. (c) Describe the different forms it

assumes. .... (d) What kind of movement are you observing? ....
Now use a pipette to transfer *Daphnia* to the slide containing the hydra. Study the behavior of the animal under low power. (e) Describe what happens. ....

....

(f) What kind of movement are you observing? ....

Now, while you are looking at hydra through the microscope, jar the slide on the microscope stage. (g) What happens? ....
Remove the cover glass and observe through the microscope what happens when you touch the tentacles, body, and basal disk. (h) Record the behavior of the hydra. ....

....

(i) You contacted only one or a few cells. How do impulses travel from one cell to another?

....

Put a drop of weak acetic acid at one side of the concavity. Observe the tentacles under high power as the acid diffuses through the water. (j) Record your observations. ....

....

Put several hydras in a watch glass and shine a light on one side. (k) Record what happens. .... Cover a flashlight with blue cellophane paper. Shine this light on one side of the watch glass and shine a white light on the other. (l) Record your observations. ....

....

Select a hydra with buds. These may be in several stages of development. Mount it on a culture slide. Put a drop of carmine red at the edge of the concavity and observe the bud and parent as the stain diffuses into the water. (m) Record your observations. ....
.... (n) Could a bud live independently of the parent?
.... (o) Explain your answer. ....
.... (p) How does the hydra reproduce other than by budding? ....

....

A Part 2 STUDY OF AURELIA. *Aurelia* belongs to the same class of coelenterates as the large jellyfish. It is found in waters all over the world. It ranges in size from 3 to 12 inches in diameter. Since the medusa stage in the life cycle is the most conspicuous, you will use it for your study of *Aurelia.*

Materials. Preserved *Aurelia;* culture dish; probe; hand lens; stereoscopic microscope.

Procedure and observations. Float your specimen of *Aurelia* dorsal side up in a culture dish. Note the circular shape and the short *tentacles* arranged close together around the umbrella. Count the *notches* in the margin of the umbrella. (a) How many do you find? ....
Use a hand lens or stereoscopic microscope to examine the *sense organs* which lie in the marginal notches. These contain a light-sensitive pigment and a sac with hard particles that func-

tion in equilibrium. Touch the medusa with your probe. (b) What is the consistency of the specimen? .... (c) Of what value is this consistency to the living organism? ....

(d) Do you find a supporting framework? ....
Examine the *nerve net* with your hand lens or stereoscopic microscope. (e) How does it differ from that of the hydra? ....

....

Turn the specimen over to expose the ventral side. Locate the *mouth* which lies in the center of the medusa. (f) What is its shape? .... Note the *oral lobes,* or *mouth lobes,* that extend from the corners of the mouth. (g) How many oral lobes do you find? .... Use your probe to examine the structure of the lobes. Then examine them with your hand lens or stereoscopic microscope. (h) Describe the structure of the oral lobes. ....

....

....

Insert the probe into the short *gullet* and then into each of the four *gastric pouches.* The horseshoe-shaped *gonads* by which *Aurelia* is recognized lie on the floor of the gastric pouches. Sexes are separate and sperm are discharged through the mouth opening. The eggs are fertilized in the gastric pouches, and are held in the mouth lobes during early development. When the young are released, they are in the form of the planulae larvae that begin the asexual stage of *Aurelia.*

Structure of *Aurelia*—ventral view

Draw the ventral side of *Aurelia* in the space provided and label: **marginal notch, tentacles, marginal sense organ, mouth, oral lobe, gastric pouch, gonad.**

B Part 3 REGENERATION IN HYDRA. The name "hydra" was first given to this organism because of its resemblance to the mythical nine-headed Hydra that could grow two heads in the place of one that had been cut off. The hydra is one of the most widely used of animals in the study of regeneration.

Materials. Hydra culture; three Syracuse watch glasses; razor blade; hand lens or stereoscopic microscope.

Procedure and observations. Transfer a well-fed hydra to a watch glass containing pond water. With a razor blade make a transverse cut through the animal. Cover the dish to prevent evaporation and place it in diffuse light in your laboratory. Examine the dish daily, and record any evidence of regeneration. (a) When did you observe the first evidence of regeneration? ....

.... (b) How long did it require your specimen to regenerate the missing halves?

....

Divide another animal into several parts. Observe these carefully each day for several days. (c) Do they all regenerate their missing parts? ....

Using a third animal, split the anterior end in two and separate the two parts. (d) Observe these each day and record what happens. ....

Interpretation. Discuss the manner in which each part of the hydra regenerates. ....

....

....

Platyhelminthes—The Flatworms

Purpose To study two common flatworms, to compare the life of the free-living one with that of the parasitic species, and to study regeneration in Planaria.

The members of the phylum Platyhelminthes are the first animals in the phylogenetic tree to have a mesoderm as well as an ectoderm and endoderm. Furthermore, they are the first animals to be built on an organ-system level of development. Flatworms do not possess all the organs found in higher forms, however, nor are the ones they possess well developed. Flatworms are also the first animals with a distinct head with eyes, sense organs, and a central nervous system. They have a distinct anterior and posterior end and a dorsal and ventral surface.

A Part 1 PLANARIA—THE "CROSS-EYED WORM." This common flatworm is found on water plants in ponds, under stones in many streams, and along the shores of lakes and rivers.

Materials. Living planarians; prepared slides of longitudinal section of a planarian; prepared slides of cross section of a planarian; microscope; hand lens; collecting jar; Syracuse watch glass; pipette; dissecting needle; culture dishes; liver; acetic acid.

Procedure and observations. If you are collecting some planarians for study, visit a slow-moving stream or pond and turn over stones and sticks in the water. Examine the undersides carefully or these animals will escape your notice. Usually planarians are found in clusters. They may appear at first as small droplets of gray, brown, or white jelly, but when transferred to a collecting jar, they will begin to crawl along the sides and bottom. Large worms may be ½ in. by ⅛ in. wide. Planarians may be cultured in a dark place in the laboratory. Remove them from the collecting jar with a pipette and put a few in each culture container with fresh water and some stones, plants, and dirt. They should be fed once a week by putting a very small piece of liver in the water, after which the water should be changed. For examination, put one planarian in a Syracuse watch glass with a little fresh water.

Examine the animal with a hand lens.

(a) Describe the dorsal surface. ……………………
…………………………………………………… Watch the animal as it crawls about in the watch glass. (b) Describe its movement. ……………………………………
……………………………………………………………………………………………………

Planaria—dorsal view

Use a pipette to create a current in the water in the watch glass. (c) How does the animal react? …………………………………………………………… The projections on the side of the head are called *auricles*. Touch them with a dissecting needle. (d) How does the animal respond?

.... (e) Touch other areas of the body and describe the reaction.

.... Note the two eyespots on the head. Shine a flashlight on the eyespots. (f) What kind of response does the planarian make? ....
Shine the light on other areas of the body. (g) Record the behavior of the animal.

....

....

Shade one area of the watch glass and shine a light on the animal. (h) What kind of response does the animal make? ....

.... Put a small piece of liver in the water and watch the animal. (i) Record what happens.

....

Put a drop of acetic acid in the water near your specimen. (j) How does it react? ....

.... Draw the dorsal view of the worm and label: **anterior, posterior, right, left, head, eyespot, auricles.**

Planaria—longitudinal section (L.P.)

Turn the animal over and examine the ventral side with your hand lens. (k) Describe the ventral surface. .... Locate the mouth and pharynx.

Examine the longitudinal section of the worm under low power of the microscope. Draw the longitudinal section in the space provided and label: **pharynx, gastrovascular cavity.**

Examine a cross section under the microscope. Draw the cross section as seen under low power and label: **ectoderm, mesoderm, endoderm, cilia.**

A Part 2 THE TAPEWORM.

The tapeworm is a long, flat, ribbonlike animal that has become adapted for life as a parasite. It has lost many of the structures found in the planarian and has become dependent upon a passive transfer in food to new hosts.

Materials. Preserved specimen of tapeworm; prepared slide of tapeworm; microscope; charts showing life cycle of tapeworm.

Procedure and observations. Examine a preserved specimen or a prepared slide of the tapeworm, if available. If not, use the diagram in Fig. 28-1-a and any available charts. Note the head, or *scolex*, with hooks and suckers. (a) What is the function of the hooks and suckers?

Planaria—cross section (L.P.)

....

The many sections that extend from the neck and form the body of the tapeworm are the egg cases, or *proglottids.* (b) Is there any evidence of a digestive system in the tapeworm?

.... (c) Explain your answer. ....

....

(d) How is a tapeworm transferred to a new host? ....

....

(e) List the ways in which the tapeworm has become degenerate. ....

....

....

(f) Which system is the most highly developed in the tapeworm? ....

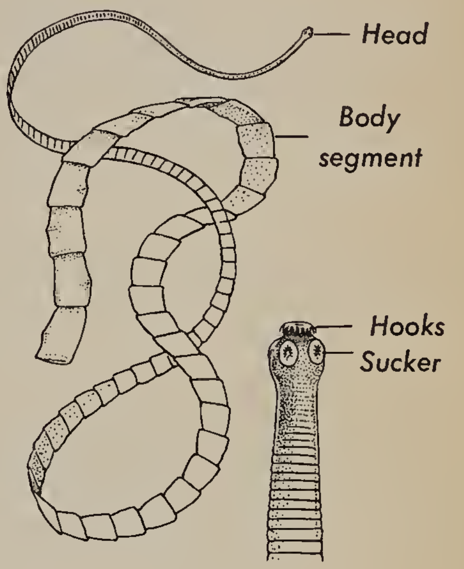


Fig. 28-1-a The tapeworm

B Part 3 REGENERATION IN PLANARIA. You have already studied regeneration in the sponge and the hydra and observed that they are able to develop an entire animal from a small section. Because planarians also have the ability to regenerate complete worms from very small pieces of body tissue, they have been used extensively for studies in regeneration.

Materials. Living planarians; microscope; slides; cover glasses; toothpicks; culture dishes; scalpel or razor blade; Epsom salts; hydrochloric acid.

Procedure and observations. Put a planarian in a culture dish and add a few crystals of Epsom salts. In a few minutes the worm will be anesthetized and can be transferred to a drop of water on a glass slide with a toothpick. Fig. 28-1-b shows various ways in which the animal may be sectioned. With a razor blade, cut the planarian in the desired location and transfer each to a separate culture dish. Add fresh water and put the dishes in a dark place. Observe the animals daily, changing the water as you do so. Make drawings of your observations in the space provided and record the time of regeneration in the table.

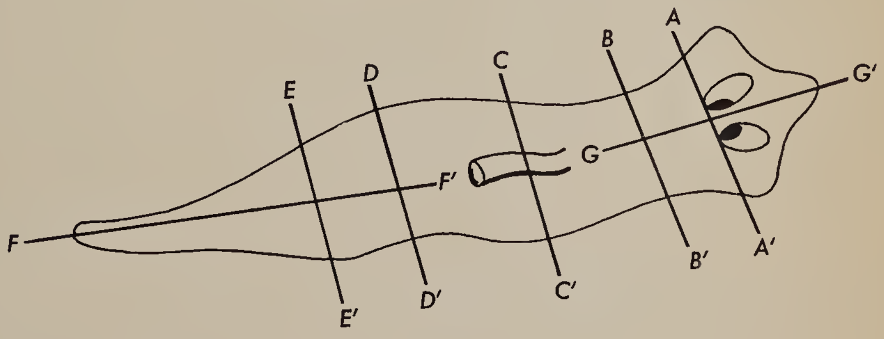


Fig. 28-1-b Various ways to section the planarian

(a) Which sections of the animal regenerated most quickly? ....

.... (b) most slowly? ....

.... (c) How does regeneration at the anterior end differ from regeneration at the posterior end? ....

Observations on regeneration

Section	*Time for Regeneration*		
	Anterior part	*Middle part*	*Posterior part*
A-A′			
C-C′			
D-D′			
A-A′ and B-B′			
D-D′ and E-E′			
	Right half	*Left half*	
G-G′			
F-F′			

.... (d) How does the regenerated growth differ from the original section? ....

.... (e) Did all of the sections produce heads?

....

(f) If not, which one or ones were unable to regenerate a head? ....

.... (g) Account for the inability of certain sections to regenerate heads. ....

(h) In what way might you produce a planarian with a head at either end or a tail at either end? ....

Interpretation. On the basis of your observations of regeneration in planarians, discuss the relative ability of the various sections to regenerate normal parts. ....

....

....

C Part 4 THE EFFECT OF COLORED LIGHT ON REGENERATION IN PLANARIA

Problem. Does colored light inhibit or stimulate regeneration in a planarian.

Planarians, like other organisms, depend on a favorable environment for survival. They are normally found in shaded areas. In Part 3 of this Investigation you studied regeneration of the animals under laboratory conditions. Does exposure to colored light affect their ability to regenerate? You can find the answer to this question by sectioning the animals and covering them with various colors of cellophane paper.

Suggested materials. Culture of planarians; stereoscopic microscope or hand lens; colored cellophane paper (yellow, red, blue, green, and clear); graph paper (marked in 0.1-mm squares); scalpel or razor blade; Syracuse watch glasses.

Suggested procedure. Cut a planarian into 4-mm sections. Put each section in individual Syracuse watch glasses and cover them with pieces of cellophane paper. Be sure to cover one with plain paper to serve as a control. Keep the animals in a cool, dark place. Examine them daily for two weeks and note any evidence of regeneration. Measure the sections to determine whether they increase in length or breadth.

Analysis and conclusions. Summarize your results in drawings and a graph. On the basis of your observations, you should be able to draw some definite conclusions about the effect of colored light on regeneration in a planarian.

Nematoda—The Roundworms 28-2

Purpose To become familiar with four common roundworms.

The roundworms are among man's worst enemies. They are found in the sea, in fresh water, and in the soil. Although many are parasites, some are free living and considered beneficial to man.

Many of the roundworms undergo complex life cycles. In this Investigation you will study four of the more common roundworms and become more familiar with the adaptations for the parasitic way of life.

A Part 1 ASCARIS. *Ascaris* is a parasitic worm found in the intestine of pigs and other vertebrates. Its anatomy is similar to that of almost any other roundworm. Its elongated cylindrical body is pointed at both ends. The sexes are separate, the female being the larger of the two.

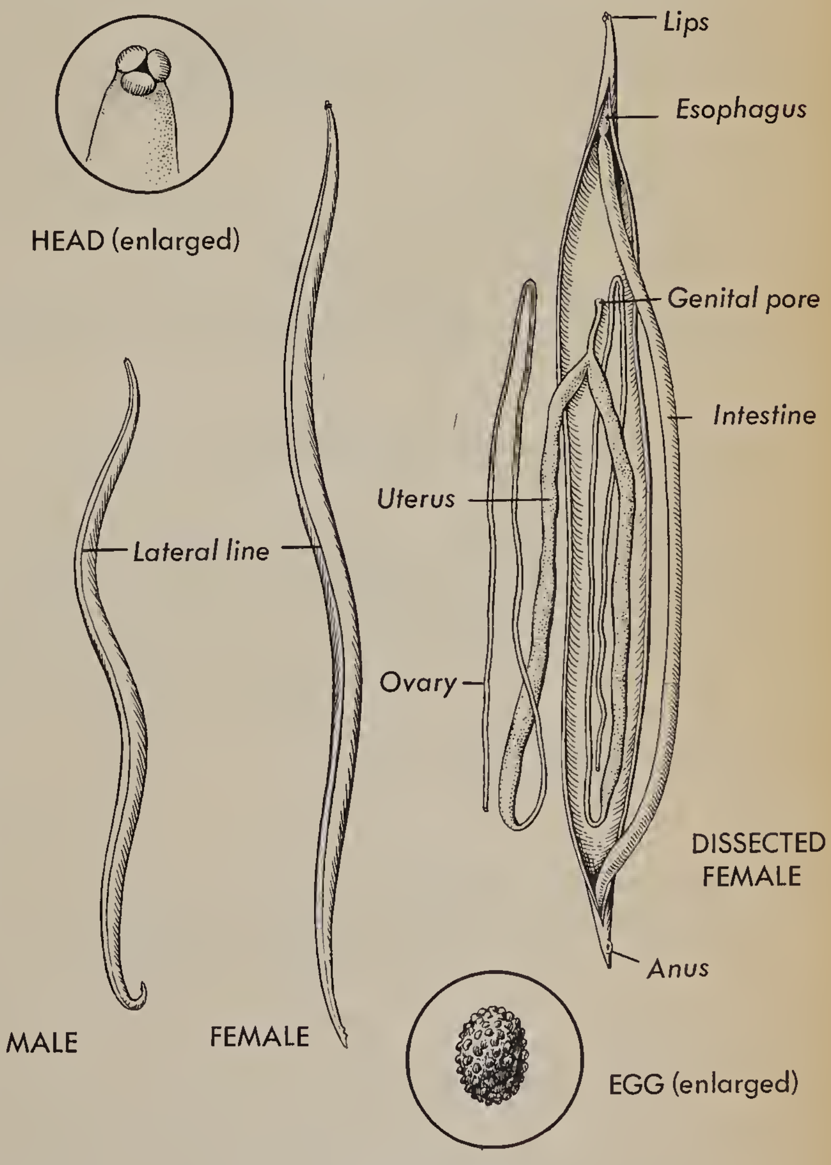


Fig. 28-2-a *Ascaris*

Materials. Preserved *Ascaris* or Fig. 28-2-a; scissors; dissecting pan; pins; dissecting needles.

Procedures and observations. Examine the specimen you have carefully. (a) Are cilia present? .... (b) Are sense organs present? ....

To reveal the internal organs, make a slit along the mid-dorsal line from the anterior to the posterior end. Be careful not to cut too deeply. Pin the sides of the worm to the pan so the internal organs are seen. Identify in Fig. 28-2-a all the structures seen. (c) In which direction do the muscle fibers run? .... (d) Is the body divided into segments? .... (e) What forms the visible lateral line? .... (f) How can a male be differentiated from a female? ....

(g) Trace the life history of an *Ascaris* from the egg until an adult that produces another egg develops. ....

....

....

(h) How are the systems of *Ascaris* modified for a parasitic way of life? ....

....

....

....

....

A Part 2 THE HOOKWORM. The tiny hookworm is much more harmful to man than *Ascaris.* It causes anemia, lack of energy, and sometimes retarded growth.

Materials. Prepared slide of the hookworm; microscope.

Procedure and observations. Examine a prepared slide of this worm. (a) How does it enter the body? .... (b) In what way is harm done to the body? .... (c) How can hookworm disease be prevented? .... (d) In what sections of the country are hookworms commonly found? ....

A Part 3 TRICHINA WORM. Trichina worms are microscopic and are very numerous in the bodies of the animals in which they are parasitic. Infestation with trichina worm is called trichinosis.

Materials. Prepared slide of trichina; microscope.

Procedure and observations. Examine a prepared slide of trichina, if available, or study Fig. 28-2-b. Notice the characteristics that resemble *Ascaris* and hookworm. (a) In what animal do the worms pass the first stage of their life history? .... (b) In what tissues of the host animal do they live? .... (c) How do they enter the human body? .... (d) How are they protected in host tissue? .... (e) How can trichinosis be prevented? ....

....

....

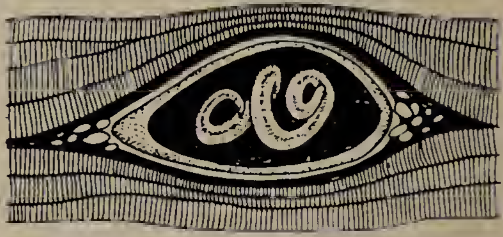


Fig. 28-2-b Trichina encysted in muscle

The Earthworm—A Representative Annelid 28-3

Purpose To study the anatomy of the earthworm and the functions of its parts, to study its responses to stimuli, and to study regeneration in the earthworm.

While the annelids are worms, they have little in common with other worms you have studied except the name. All annelids have segmented bodies, and the segmentation is internal as well as external. Segmentation was important in evolution because it permitted an increase in division of labor between the different regions of the body. Another structural innovation in the annelids is a closed circulatory system. The coelom, a cavity between the body wall and alimentary tract, first appears in the annelid worms. This cavity permits an animal such as the earthworm to grow larger in size than a planarian or a roundworm.

A Part 1 STRUCTURE OF THE EARTHWORM. The common earthworm, sometimes called nightcrawler, has nearly all the organ-systems found in higher animals–integumentary, muscular, digestive, circulatory, excretory, reproductive, and nervous. In this Part you will perform your first dissection to determine the anatomy of these systems.

Materials. Earthworm (preserved or living); microscope; prepared slide of cross section of earthworm; hand lens or stereoscopic microscope; dissecting pan; scissors; scalpel; forceps; dissecting needles; probe; straight pins.

Procedure and observations. Section 1 *External structure of the earthworm.* Examine your specimen carefully. Rub your fingers over the surface. (a) Describe the body covering. .... The bristles on the body surface are called *setae.* (b) How many are there on each segment? .... (c) Where are they attached? .... (d) How do they function in locomotion? .... (e) Determine whether the animal has a definite anterior and posterior end and explain your answer. .... Locate the *mouth* and the *anus.* (f) What is the function of the anus? .... Locate the *prostomium* above the mouth. Use a hand lens or stereoscopic microscope to locate the *excretory pores.* (g) How many are there on each segment? .... (h) What is the function of the excretory pore? .... Examine segment 14 and locate the *female pore,* through which eggs leave the body. Locate the *male pore* on segment 15. Sperm leave the body through this opening. Locate the openings on segments 9-10 through which sperm enter the worm. Examine the thickened area encircling the body. This is the *clitellum.* (i) What is its function? ....

Section 2 *Internal structure of the earthworm.* Using a preserved earthworm, observe the following instructions in your dissection of the animal.

1. Read the directions carefully *before* you begin to cut.
2. Identify structures to be cut *before* cutting.
3. Lift the structures to be cut.

4. Cut only when directed to do so.
5. When possible use your fingers instead of a needle to expose structures.
6. Before you begin be sure you understand the meaning of the following terms: *anterior, posterior, dorsal, ventral, median, longitudinal.*
7. Drawing and labeling should be carried on as you investigate each structure. Begin by making a large outline in the space provided on page 253; then add structures and their labels as you observe them. Label all structures in **boldface** type. Use a sharp pencil and make your diagram neat and concise.

Put the specimen in a dissecting pan with the dorsal side up. Pin the anterior and posterior parts to the pan, using care not to put the pins through any internal organs. Use your scissors to cut through the body wall slightly to the left of the mid-dorsal line. Begin about an inch behind the clitellum and extend the cut to the mouth. Be careful not to cut through anything but the body wall. Separate the edges of the cut and look into the body cavity. Observe that the body wall is separated from the intestine (the long brown structure) by a definite space called the **coelom.** Notice that in the coelom there are **septa** (partitions) extending from the body wall to the intestine. Using forceps and needles, carefully cut the septa, segment by segment, until the anterior end of the worm is entirely open for study. Pin down the sides of the body wall as you cut. You are now ready to begin examining particular systems of the earthworm.

A. Muscular system. Locomotion in an earthworm is accomplished by two sets of opposing muscles. The **circular muscles,** lying just beneath the body wall, form rings parallel to the circumference of the worm. When these muscles contract, the segments are constricted, causing the worm to lengthen. Another muscle layer, the **longitudinal muscles,** lie just beneath the circular layer. These muscles lie parallel to the length of the worm. When the longitudinal muscles contract the body shortens, causing the segments to thicken. If portions of the worm are anchored by setae, the unanchored portions move toward the anchored region. (j) Explain how the worm moves if the anterior end is anchored. ....

....

B. The digestive system. This system is composed of a tube extending from the mouth to the anus. This digestive tract has specialized areas which you will observe and include in your diagram. The **buccal cavity** is a small tube which passes from the mouth to about the third segment. The **pharynx** is the thick-walled area posterior to the buccal cavity. (k) What is the purpose of thick walls? .... (l) What is the function of the pharynx? ....
The **esophagus** is a long, slender tube extending from behind the pharynx to segment 15. (m) What function does the esophagus serve? ....
The **crop** is the large and thin-walled area posterior to the esophagus. (n) What is its function? .... The **gizzard** is the thick-walled area posterior to the crop. (o) What action occurs in this structure? ....

.... You have already identified the **intestine,** which extends from the gizzard to the anus.

C. The reproductive system. In segments 10, 11, and 12 there are pairs of white structures called **seminal vesicles.** Sperm are stored in these organs. In segments 9 and 10 there are pairs of small, white spherical structures called **seminal receptacles.** These organs receive sperm from another earthworm. Under the seminal vesicles you may be able to locate the **ovary,** in which eggs develop. Review your study of external structure to recall the outside openings of these reproductive structures. (p) Referring to your textbook, explain how earthworms repro-

Internal structures of the earthworm

duce, naming the structures you have observed. ....

....

....

D. The circulatory system. The **dorsal blood vessel** appears as a dark brownish vessel running along the medial surface of the intestine. In many individuals you can see or the intestinal wall the two pairs of branches which this vessel gives off in each segment. Remove the seminal vesicles from the left side of the body. Look in segment 11 for a pair of stout tubes arising from the dorsal vessel and extending ventrally. These "hearts," or **aortic arches,** are often discolored because they contain blood. (q) What is the function of these structures?

....

Now look in each segment from 11 forward to 6 for a similar pair of tubes. You will have to remove the septa to see these clearly. Use your dissecting needle to push the intestine to the right side to enable you to see the median ventral line. You should then be able to see the brownish **ventral blood vessel.** Follow this vessel to its connection with the aortic arches.

(r) Trace the course of blood in the earthworm. ....

....

....

E. The excretory system. The coiled white, loose tubes along each side of the digestive tract are the excretory organs, called **nephridia.** They are located in each segment except the first three and the last. In order to see the nephridia better, extend the body wall incision back about two inches. Open the body wall and pin it back in this area. Carefully pull out the intestine from the body cavity. Observe the nephridia under the dissecting microscope, using your dissecting needle to search them out. (s) How are the nephridia connected to the outside?

.... (t) To what organs in man do

the nephridia correspond? ....

F. The nervous system. In the area of segments 2 and 3, dorsal to the buccal cavity, the "brain," or **suprapharyngeal ganglion,** is located. Two nerve fibers arise from the suprapharyngeal ganglion. Each passes around the pharynx and joins the large ganglion below the pharynx, called the **subpharyngeal ganglion.** From this ganglion the **ventral nerve cord** extends the length of the animal. Remove part of the intestine and locate the nerve cord. Note the small ganglion at each segment. Cut the nerve cord and pull it free from the body. Now observe the small nerves branching from the ventral nerve cord. (u) Where do these nerves go? ....

.... (v) What is their function?

Cross-sectional view of an earthworm

....

There is no evidence of a specialized respiratory tract. (w) Explain how oxygen and carbon dioxide are exchanged in the earthworm. ....

Examine the cross section of the earthworm under the compound microscope. Study the structures carefully and draw the cross section in the space provided. Label: **circular muscle, longitudinal muscle, dorsal blood vessel, epidermis, intestine, cuticle, seta, body cavity** (coelom), **ventral nerve cord, ventral blood vessel, nephridium.**

A Part 2 ACTIVITY AND RESPONSES OF THE EARTHWORM. Since the earthworm has a more highly developed nervous system than the animals you have studied previously, you would expect it to have better coordinated responses. Although the animal has no eyes and ears, it has receptors for light and vibrations in its skin, as well as a small brain and nervous tissue in each segment.

Materials. Living earthworm; dissecting pan; paper toweling; probe; flashlight; red light source; moist soil; celery leaves; carrot leaves.

Procedure and observations. Dampen a piece of paper toweling and put it in the bottom of a dissecting pan. Put a live earthworm on the damp paper and observe its locomotion. (a) Describe the movement in the earthworm. .... (b) In terms of what you know about its muscles, explain why the earthworm moves in this manner. ....

....

Observe the dark-colored structure that extends the length of the dorsal surface of the animal. This is the dorsal blood vessel. Watch the flow of blood through this vessel. (c) In what direction does it flow? .... (d) Does the earthworm have any visible sense organs? ....

Using a probe, touch the head end and then other areas of the body. (e) What kind of response did the animal make? ....
(f) Which area or areas is most sensitive to touch? ....

....

Shine a flashlight on the anterior end of the worm. (g) Describe the reaction. .... (h) Explain how this behavior is related to survival. .... Shine the flashlight on other areas of the body. (i) Describe the reactions. .... Shine the red light on the anterior end of the worm. (j) Describe the reaction. .... (k) Is the reaction the same as that made in response to the white light of the flashlight? .... (l) Explain your answer. ....

Put some celery leaves in one end of the dissecting tray and carrot leaves in the other end.

Watch the animal carefully. (m) Record its behavior. ....

....

Put your specimen on top of loose moist soil. (n) Observe and record its behavior. ....

....

(o) How is this behavior related to soil fertility? ....

....

C Part 3 REGENERATION IN THE EARTHWORM

Problem. To what extent can the earthworm regenerate tissues?

As the degree of tissue specialization in an animal increases, the ability to regenerate lost parts decreases. Can the earthworm, an animal with a high degree of specialization, regenerate an entire body from a few segments?

Suggested materials. Living earthworms; Syracuse watch glasses; hand lens; scalpel or razor blade; alcohol or ether (to anesthesize the worm).

Suggested procedure. Cut a healthy earthworm into pieces of ten segments each. Put each section in a separate, clean Syracuse watch glass. Identify the segments as to number in the earthworm's body. Keep the sections at 50°-60°F. Observe them at 24-hour intervals for three weeks. Look for the development of an anus and changes in the ectoderm and endoderm. Note any evidence of a head or tail. Measure the sections to determine whether there is increase in length.

Analysis and conclusions. Summarize your data as drawings to show any changes in the various sections. Your observations should enable you to draw some definite conclusions about the ability of specialized tissues such as those found in the earthworm to regenerate.

Purpose To study representatives of the Phylum Mollusca and to determine the rate at which a snail travels.

The mollusks afford an excellent example of adaptive radiation. Through the course of evolution the basic body plan of the first members of the phylum has become modified into several different forms as the animals spread to different environments. All mollusks have a fleshy mantle and a dorsal visceral hump and must have a muscular foot. They vary considerably, however in size, type of shell, and the way they feed.

A **Part 1** **CHARACTERISTICS OF THE MOLLUSKS.** The mollusks are separated into classes on the basis of their symmetry, the nature of the foot, the mantle, the gills, and the radula. In this Part you will study four representatives of the phylum to determine their characteristics.

Materials. Preserved specimens of chiton, clam, snail, and squid; charts of chiton, clam, snail, and squid; hand lens; dissecting tray; dissecting needle; probe.

Procedure and observations. Examine the specimens or charts or use the diagrams in Fig. 29-1-a. Determine whether a valve is present, the type of symmetry, whether the animal is segmented or not, whether jointed appendages are present, the type of foot, and whether there is a mantle present. Record your information in the table provided.

Characteristics	*Clam*	*Snail*	*Chiton*	*Squid*
Valve or shell				
Symmetry				
Segmentation				
Jointed appendages				
Foot				
Mantle				

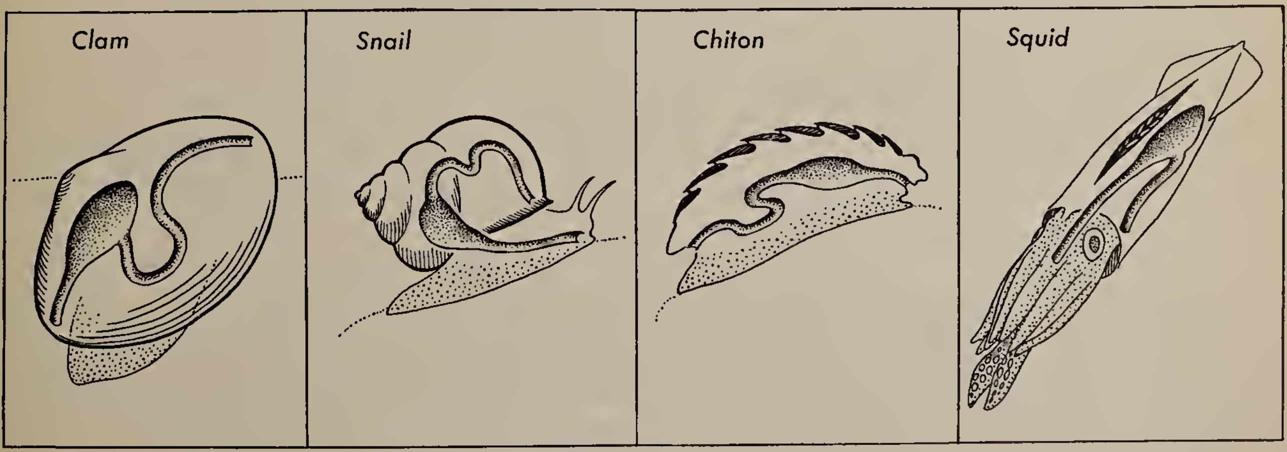


Fig. 29-1-a Four mollusks

A **Part 2** **ANATOMY OF THE CLAM.** The clam is a bivalve along with the oysters, scallops, and mussels. Its heavy shell does not allow it to move around much, but affords maximum protection. The incoming and outgoing siphons that carry water adapt the clam to its method of trapping food in mucus.

Materials. Preserved specimens of clams; clam charts and diagrams; dissecting tray; scalpel; probe; dissecting needles; scissors; screw driver.

Procedure and observations. Examine your specimen and note that the shell consists of two parts, called *valves.* The shells are fastened together along the dorsal surface by an *elastic ligament.* Locate the elevated hump, the *umbo,* that is near the anterior end of the ligament. This is the oldest part of the shell. As the clam grows, it secretes additional layers, each one extending beyond the last one laid down. This produces a series of concentric *annual growth rings* which you can locate on the shell. Note that the outer portion of the shell is a thin, dark, and *horny layer.* It is often lacking on the umbo of older specimens. The middle layer, or *prismatic layer* is formed of crystals. Examine the inner layer, or *pearly layer.* Using your probe, locate the two openings on the posterior end. The ventral one is an *incurrent opening* for the entrance of water currents, and the dorsal one is the *excurrent opening,* through which currents of water leave the clam. The shells open on the ventral surface.

Use your screw driver or another flat instrument to open the shells. Cut the two large muscles that hold the shell closed. These are the *anterior* and *posterior adductor muscles.* Open the shell and lay it back on the dissecting tray. Note the *scars* that are left by the removal of the muscles. Examine the dorsal edge of the shell and note the toothlike projections that fit into the grooves in the opposite shell. (a) What purpose does this arrangement serve? ....

.... Examine the interior of the shell, noting the smooth, glistening appearance. This is the third layer, or *pearly layer,* of the clam shell. (b) Why is the interior of the clam shell smooth? ....

....

Locate the visceral mass that lies in the dorsal region between the two large adductor muscles. Examine the *mantle* covering the visceral mass and note that it extends ventrally, forming two mantle lobes, one just inside each shell. The space between the two lobes is the *mantle cavity.* Remove a mantle lobe and expose the mantle cavity and its organs. The muscular *foot* extends into the mantle cavity and out between the shells. Locate the pair of sheetlike *gills* that hang into the mantle cavity on either side of the foot. Use the hand lens to find the *cilia* on the gills. The beating of these cilia draws water into the incurrent opening. Locate the *mouth* and the pair of folds, the *palps,* on either side of the mouth. Insert the probe into the mouth and direct it into the *esophagus* and into a saclike stomach.

Use a scalpel to cut away the muscle of the foot and expose the *intestine, digestive gland* surrounding the stomach, and the *pericardial cavity* containing the heart.

If you do not have a preserved specimen, locate the italicized structures in the diagram in Fig. 29-1-b. Then label the following on the diagram: **mouth, esophagus, stomach, intestine, anus, digestive gland, mantle, heart, gills, foot, anterior adductor muscles, posterior adductor muscles, valve, incurrent opening, excurrent opening.**

A Part 3 STUDY OF THE SNAIL. The class Gastropoda, which means "stomach-foot," includes the snails, conches, and abalones. These animals lie in between the almost sessile, mucus-feeding bivalves and the fast-moving cephalopods that hunt for their food. Snails are protected by a shell that is light enough to allow them to move around to obtain food. They do not move fast, but this is not a disadvantage as they feed mostly on plants.

Materials. Living specimens of snails; preserved specimens of snails; dissecting tray; scalpel; probe; aquarium; dissecting needles.

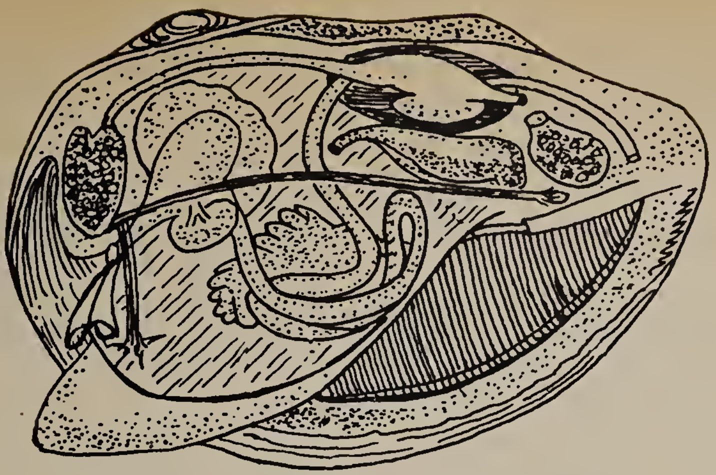


Fig. 29-1-b Anatomy of the clam

Procedure and observations. Let a snail crawl on your desk or on the dissecting tray or on the sides of an aquarium. (a) Describe the manner in which the animal moves. ....

.... Note the two pairs of *tentacles* on the anterior end of the animal. Study the base of the tentacles and find the *eyes* that are located on one pair of tentacles. Use the probe to touch the tentacles. (b) Describe what happens. ....

....

Remove the shell carefully from a preserved specimen by picking off pieces with your dissecting needles. Note the long, flat *foot* and distinct *head.* Examine the *mouth* and locate the *radula.* The *visceral hump* is also present in the snail. Locate the *mantle, mantle cavity,* and *intestine.* Make a drawing of your specimen in the space provided and label all the structures you were able to find.

B Part 4 A SNAIL'S PACE. The expression "a snail's pace" indicates very slow progress. In this Part you will attempt to measure the actual rate at which a snail moves under different conditions. Since your snail may not move at all, you may have to devise ways to prod it.

The snail

Materials. Live snails; container for snails; thermometer; metric rule; white paper; sandpaper; stopwatch; ice cubes.

Procedure and observations. Section 1 *The effect of obstacles on a snail's pace.* Draw concentric circles at 5-mm intervals on a piece of white paper cut to the size of the bottom of your container. Put the paper under the container and put water in it. Then position the snail in the center of the bowl. Observe the distance the snail travels in one minute, and record this distance in the table provided. You may have to prod the snail to start it in motion. Repeat this observation a second and third time in order to obtain an average. (It may be necessary for you to share data in this Investigation in order to complete the table.)

Mark a piece of sandpaper in the same way that you marked the white paper, and put it under the container. Repeat the timed measurements as above and record in the table.

Make an inclined plane with the metric rule and try to get the snail to move upwards on it. Make three timed measurements, record your data in the table, and compute the average. Now try to get the snail to move down the inclined plane. If you are fortunate, you may get three timed measurements and an average.

Now compute and record the snail's speed up and down the vertical surface of the container.

(a) At room temperature, how long would it take a snail to travel a mile? ....

(b) How long would it take you to walk a mile? ....

Speed of snail (mm/min.)	*Glass surface*	*Sandpaper surface*	*Up inclined plane*	*Down inclined plane*	*Up vertical surface*	*Down vertical surface*
Trial 1						
Trial 2						
Trial 3						
Average						

Section 2 *Effect of temperature on a snail's pace.* Use a thermometer to stir an ice cube in the water in the container. When the temperature has dropped several degrees, remove the ice cube and put the snail in the center of the container. (If a land snail is used, the dish may be put on ice cubes or in the refrigerator.) Observe and record the snail's rate and compute an average in the same way you did in Section 1. (c) Does this average rate differ from that at room temperature? .... (d) Explain. ....

....

Use a thermometer to stir a little warm (not boiling) water into your container. When the temperature has risen above room temperature, put the snail in the center of the dish. (If a land snail is used, the dish may be put over warm water or in an incubator.) Make timed measurements of the animal's movement and record in the table. (e) Is this average rate different from that at room temperature? .... (f) Explain. ....

....

Speed of snail (mm/min.)	*Room temperature*	*Cold water*	*Warm water*
Trial 1			
Trial 2			
Trial 3			
Average			

Interpretation. Account for the variation in rate of movement under the various temperature ranges. ....

....

....

Purpose To examine the crayfish, to study its activity and responses, and to study the effect of temperature on the heartbeat of *Daphnia.*

If numbers of individuals and species are taken as criteria of success, the Arthropoda are the most successful animals. Their body segmentation suggests that they evolved from the annelids. The segments, however, have become more specialized, and in the higher arthropods almost every segment has a somewhat different structure and function. Specialized sensory receptors such as eyes and antennae, the arrangement of the muscles in groups, jointed legs, and a protective body covering have enabled the arthropods to become more successful than the annelids.

Although arthropods vary greatly in structure, the basic body plan is the same. Thus, in your study of the crayfish, you will become familiar with the characteristics of the arthropods in general as well as those of the class Crustacea.

A Part 1 EXTERNAL ANATOMY OF THE CRAYFISH. The segmented appendages of the crayfish seem to have evolved from a common type, but have become greatly modified to carry on several different functions. The extinct trilobite, an ancestral arthropod, had paired appendages similar to the swimmerets of the crustaceans on every segment of its body. For this reason the swimmeret is considered the basic type of crustacean appendage. Appendages that are similar in origin and structure, but have a different function, are referred to as homologous structures.

Materials. Preserved specimen of crayfish; charts or diagrams of crayfish; hand lens; dissecting tray; probe; forceps; scissors; scalpel.

Procedure and observations. Put the preserved crayfish, dorsal side up, in the dissecting tray. Examine the hard chitinous *exoskeleton* and note that the body is divided into two distinct regions, the *cephalothorax* and the *abdomen.* The abdomen ends in a reduced abdominal segment called the *telson.* Note that the cephalothorax is covered by a single piece of the exoskeleton called the *carapace.* Locate the *cervical groove* that marks the division between the head and thorax. There are either 12 or 13 segments in the cephalothorax, depending on whether the segment bearing the antennules is considered a true segment. These segments are more easily located on the ventral surface. (a) How many segments are there in the abdomen? ....

The carapace extends anteriorly and forms a horny beak, the *rostrum.* Locate the stalked *compound eyes* that lie beneath the rostrum. Examine the eyes with a hand lens and note the many lenses (facets) that compose a compound eye. (b) What shape are they? ....

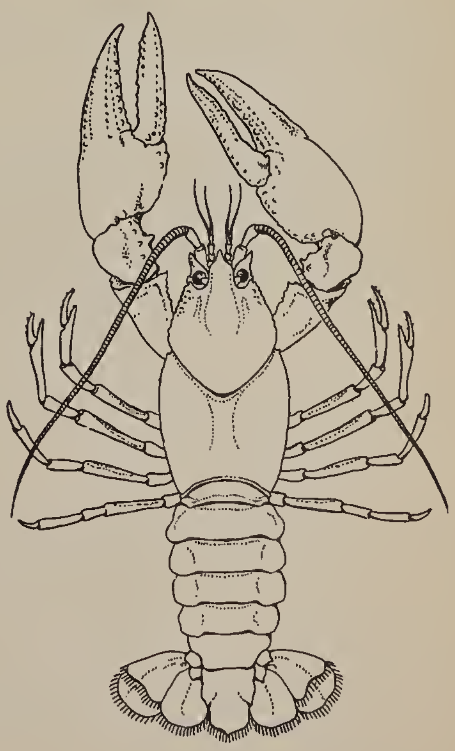


Fig. 30-1-a Crayfish—dorsal view

The most anterior appendages are the branched *antennules*. Locate the *antennae* attached just posterior to the antennules. Examine the antennules and antennae with your hand lens.

(c) Describe their structure. ....

Turn the specimen over and examine the mouth and other appendages on the head. Locate the *mandibles,* or true jaws, which lie just posterior to the antennae. Examine the *first pair of maxillae,* which are just posterior to the mandibles, and the *second pair of maxillae,* which lie posterior to the first pair. (d) How do the mouthparts of the crayfish move?

....

The thorax bears eight pairs of appendages, three pairs of *maxillipeds* and five pairs of legs. Locate and examine the *first maxillipeds,* which are posterior to the second maxillae, then the *second maxillipeds,* posterior to the first, and the *third maxillipeds,* posterior to the second. (e) How do the first maxillipeds differ from the second and third maxillipeds?

....

(f) How do the second maxillipeds differ from the third maxillipeds? ....

....

(g) How do the third maxillipeds differ from a walking leg? ....

....

The next pair of appendages are the most obvious structures on the thorax. These are the *chelipeds.* Note the well-developed pinchers that are formed on the chelipeds. (h) Are they alike? .... (i) If not, how do they differ? ....

....

The next four pairs of legs are called *walking legs.* Examine them carefully. (j) In what ways do they differ from one another? ....

The abdominal appendages of the crayfish are called *swimmerets.* The first pair of swimmerets in the female are reduced in size. In the male the first two pairs are modified for transferring sperm. (k) What is the sex of your specimen? ....

The sixth pair of swimmerets is greatly modified to form the *uropods,* which together with the telson form the powerful tail fin used in backward swimming. Locate the *anal opening* on the ventral side of the telson.

Label the following on the diagram in Fig. 30-1-a: **rostrum; antennule; antenna, compound eye, cephalothorax, abdomen, cheliped, uropod, flipper, walking leg.**

Carefully remove an antenna, maxilliped, walking leg, and swimmeret with your forceps. Straighten these out on a sheet of paper and draw them in the space provided.

Antenna Maxilliped Walking leg Swimmeret

A Part 2 **ACTIVITY AND RESPONSES OF THE CRAYFISH.** The sense organs of the crayfish are more highly developed than those in the annelids. The chemical receptors responsible for both taste and smell are located in the antennules. The hearing apparatus is located in the "ear" sac. Sensory bristles all over the body are sensitive to touch.

Materials. Live crayfish; aquarium; probe; dissecting pan; ink; beef broth; rock small enough to put in aquarium; salt solution; conc. sugar solution or vinegar.

Procedure and observations. Put a rock in the bottom of an aquarium, cover it with water and then put a living crayfish in the aquarium. Allow the crayfish to adjust to the new surroundings for five minutes. (a) Observe and record the behavior of the crayfish during this period. ....

(b) Describe the manner in which a crayfish walks. ....

....

Use the probe to touch the antennae. (c) How does the crayfish react? ....

....

Use the probe to touch other structures. (d) What structures are especially sensitive to touch?

....

Shine a flashlight on the anterior end of the crayfish's body. (e) How did this crayfish respond? ....

Now shine the light on the posterior end of the animal. (f) Describe the reaction. ....

.... (g) Explain this behavior. ....

....

Put some meat juice in the water near the animal. (h) Describe the behavior of the animal.

....

Add a few drops of a saturated solution of table salt to the water. (i) Record the behavior of the crayfish. ....

....

You may also use a sugar solution or vinegar to determine the reactions of the animal. (j) Was the reaction to these substances negative or positive? ....

Clean the aquarium and add a drop of ink just posterior to the cephalothorax. (k) What is the direction of the flow of water through the branchial chambers? ....

.... (l) What creates these water currents? ....

....

Remove the crayfish from the aquarium and put it in a dissecting pan or on a piece of moist paper toweling. (m) Does the animal right itself immediately? ....

(n) Describe the manner in which it rights itself. ....

.... (o) From your reading in the text, tell what structure is responsible for this behavior? .... (p) How does it operate?

....

A Part 3 **INTERNAL ANATOMY OF THE CRAYFISH.** Many of you have eaten lobster, a close relative of the crayfish, and are familiar with the muscles, which occupy much of the body. They are arranged by segments to provide movement of the various appendages. In this Part you will examine the other organ-systems of a crustacean.

Materials. Preserved crayfish; dissecting tray; scalpel; scissors; probe; dissecting needles.

Procedure and observations. Read the following directions carefully before starting your dissection. Each of the main organs which you are to find is indicated in *italics* in the directions. When you have completed your dissection, draw a dorsal view in the space provided and label the **heart, esophagus, stomach, intestine, reproductive glands and ducts, digestive organs, brain, nerves, nerve cord, ganglia,** and **kidneys.**

Put the animal in the dissecting pan dorsal side up. Carefully insert the point of the scissors under the top of the shell at the back of the cephalothorax and cut up the midline to the rostrum. Cut across the carapace just back of the eyes and remove the two pieces of the carapace. Note the exposed gills and study their structure. Remove the gills and legs attached to the thorax. Carefully separate the dorsal layer of muscles in the thorax and locate the light-colored *heart* just underneath. Remove the heart and cut out the sides of the thorax to expose the organs underneath. The two light-colored masses extending on each side of the body into the head are the *digestive glands.* Between these will be found the small pair of white *reproductive organs* and *ducts* in the male animal. The female will probably have a large mass of dark-colored *eggs* in the body which will have to be cleaned out before proceeding further. This will destroy the reproductive organs of the female. To locate the *intestine,* insert the point of the scissors under the dorsal side of the shell of the abdomen and cut back to the telson. Spread the shell, and the intestine will be found as a tube on the top side of the muscles of the abdomen. Trace it forward to the point where the intestine joins the large, thin-walled *stomach* in the front part of the cephalothorax.

Now remove all the organs in the thorax by cutting the short *esophagus* below the stomach and the bands of muscle holding the stomach just back of the eyes. You should be able to lift out most of the internal organs in one piece. Clean out the remaining tissue in the head so that the *green glands,* or kidneys, just posterior to and below the antennules, are exposed. In the front part of the head cavity, between the eyes, note the small mass of white tissue, the *brain.* Trace the *nerves* that go from the brain to the antennae and the eyes. Cut the hard tissue on the floor of the thorax with your scalpel so that you can follow the *nerve cord* back from the brain to the abdomen. Spread the shell of the abdomen apart and pull out the large muscle. (This is the part of the body eaten in shrimp and lobster.) The nerve cord is now exposed on the floor of the abdomen. The enlargements of the nerve cord in each segment of the abdomen are the *ganglia.*

Internal organs of the crayfish (dorsal view)

B Part 4 BEHAVIOR OF TERRESTRIAL CRUSTACEANS. Although most crustaceans are marine, sowbugs and pillbugs are terrestrial. In this Part you will study the behavior patterns that enable these animals to live out of water.

Materials. Ten live sowbugs or pillbugs; stacking culture dishes; blotters or paper towels; black paper.

Procedure and observations. Put five or six of the animals in each of two dishes. In the center of one place a wet towel or blotter. Keep the dishes away from heat or strong light and observe them for 10 or 15 minutes. (a) Are the animals scattered throughout the dishes? ....

(b) If not, where are they found? ....

If you did not note any differences, try the following: Line one half of the bottom of a dish with a damp blotter and the other half with a dry blotter. Put some animals in the dish and observe throughout the day. (c) Where do you find the animals? ....

Cut a piece of paper towel, blotting paper, or filter paper to fit the bottom of two other containers. Moisten the paper and then put five or more animals in each. Cover one half of each container with black, lightproof paper. Put one container in a closed drawer or under a box, the other in a well-lighted area. (d) Where are the animals found in each dish after 10

Line the bottom of two dishes with black, lightproof paper, cover with damp blotting paper, and put five or more animals in each. Now place a light under one side of one dish,
to 15 minutes? ....

arranging it to heat only part of the dish. (e) Describe what the animals do. ....

(f) What do they do in the other dish? ....

Interpretation. Summarize your conclusions about the reactions of sowbugs and pillbugs to moisture, light, and heat. ....

....

....

On the basis of your conclusions, when do you think these animals would search for food—night or day? ....

Do your experiments suggest the habitat of these animals? ....

....

Can you present some proof that your conclusions are right? ....

....

....

C Part 5 EFFECT OF TEMPERATURE CHANGES ON THE HEARTBEAT OF DAPHNIA

Problem. Does a change in water temperature affect the heartbeat of *Daphnia?*

Temperature is an important factor in the environment of organisms. Exposure to extreme temperatures results in injury or death. How do temperature fluctuations vary the rate of metabolic activity. You can study the effects of temperature on heart rate directly by using the small crustacean called *Daphnia,* because it is transparent.

Suggested materials. Culture of *Daphnia;* microscope; concave (culture) slides; cover glasses; thermometer; beaker; dropping pipette; timer clock or stop watch; cotton; ice.

Suggested procedure. Obtain a rich culture of *Daphnia* and examine several organisms under the low power of your microscope. Determine the location of the heart in the body of the animal. Using a dropping pipette, transfer one *Daphnia* and the water from the culture to a concave slide. Add a few strands of thread or algae to the culture. These will aid in keeping the animal from swimming out of view. Using the timer clock or stop watch count the number of pulsations of the heart for one minute. This count will serve as the control. Determine the temperature of the control.

Remove some of the water from the culture and heat it slightly so that the temperature is 5° above that of the water used for the control. Put a drop of the water and a *Daphnia* in a concave slide and count the rate of the heartbeat under low power of the microscope for one minute. Continue to heat the water and check the heartbeat of the animal at 5° intervals up to 50°C.

Put some of the water from the culture into a beaker and add ice to reduce the water temperature. Lower the temperature to 0°C, checking the heartbeat at 5° intervals.

Analysis and conclusions. How did the heartbeat fluctuate within the range of temperatures used in the experiment? What are the extremes of temperature that *Daphnia* can tolerate? Summarize your data as a graph. Your study of the influence of temperature on the heartbeat of *Daphnia* should help you understand the direct relation of external temperature to metabolic activities in a cold-blooded animal.

The Grasshopper 31-1

Purpose To examine the grasshopper, an example of the class Insecta of the phylum Arthropoda.

Since the grasshopper is not too highly specialized, biologists usually consider it representative of the class and use it for study in the laboratory.

A Part 1 **EXTERNAL ANATOMY.** Insects have the head, thorax, and abdomen separate, three pairs of legs, one pair of antennae, and usually two pairs of wings.

Materials. Preserved specimens of grasshopper; charts; dissecting pan; scissors; forceps; scalpel; hand lens.

Procedure and observations. Examining a preserved specimen carefully, locate the following structures (use hand lens when necessary). Draw a side view in the space provided and label: **antenna, compound eye, thorax, tympanum** (ear), **jumping leg, ovipositor, abdomen, spiracle, walking leg, palpus, head, simple eyes.** Stretch the wings, draw and label: **fore wing** and **hind wing.**

Lateral view of grasshopper

Fore wing and hind wing

(a) What type of food does the grasshopper eat? ....

Carefully remove each mouth part with a forceps. Draw them in the upper spaces provided. Give the function of each in the lower spaces provided.

Labrum (upper lip)	*Mandible (jaw)*	*Maxilla (food guide)*	*Labrium (lower lip)*	*Hypopharynx (tongue)*

A Part 2 INTERNAL ANATOMY. All of the systems found in higher animals are represented in the grasshopper.

Materials. Same as for Part 1.

Procedure and observations. Read the following directions carefully before starting your dissection. Each of the main organs you are to find is indicated in *italics* in the directions. When you have completed your dissection, make a drawing in the space provided and label: **esophagus, crop, gastric caeca, stomach, Malpighian tubes, intestine, colon, anus, dorsal blood vessel, dorsal ganglion, ventral ganglion, ventral nerve cord, ganglia.**

Remove the three left legs. At the dorsal surface of the last abdominal segment, make an incision in the mid-dorsal line. Continue this cut anteriorly to the front of the thorax. Be sure you do not cut too deeply. Carefully cut around the anterior part of the thorax and around the posterior part of the next-to-last abdominal segment. Slowly pull back this large "flap" in the side of the insect. The attachments of muscles and tissues to this side of the insect may be cut very close to the exoskeleton by using the scalpel. Notice the large *dorsal blood vessel.* As you reach the area of the spiracles, notice the finely branched tubes leading from them. These are the *tracheae.*

Now continue the cut over the head and carefully remove the exoskeleton in the area between the eye and the antenna. Look for a small whitish or yellowish mass. This is the *dorsal ganglion.* If you have done a careful dissection, you can find a large nerve running ventrally to a point below the esophagus where it joins another large mass, the *ventral ganglion.* Now follow the *ventral nerve cord* posteriorly for a few sections until you have seen several *ganglia.* This arrangement of a nerve cord and ganglia is often called a *gangliated nerve cord,* or *ganglionic chain.*

Beginning with the *esophagus,* cut away the tissues lateral to it and follow the digestive tract all the way through the animal to the anus.

Internal anatomy of the grasshopper

Study of a Bony Fish

34-1

Purpose To observe locomotion and other activities of a living fish, examine the external structures of a fish, and investigate protective coloration and color changes in fish under controlled conditions.

Every structure of the fish is ideally suited to aquatic life. The body is a perfect example of streamlining. Fins are ideal locomotor appendages. Gills are highly efficient organs of respiration in aquatic environments. The body covering provides the greatest amount of protection with the least resistance in the water.

A Part 1 STUDY OF A LIVING FISH. What part of the body of a fish provides the thrust to propel it through the water? How does the fish use its various fins? How is water forced over the gills? These and other questions concerning activities and behavior can be answered by observing a living fish closely. A goldfish is a good subject because it will survive in a small aquarium. However, catfish, carp, or other fish native to your region may be used.

Materials. Living fish (goldfish or other species, body length 3-5 in.); small aquarium (1-3 gal.) or battery jar (8 in. or larger).

Procedure and observations. Put a living fish in an aquarium with the water an inch or more above the dorsal fin. Allow several minutes for the fish to adjust to the surroundings. Examine the body closely and identify the head, trunk, and tail regions. (a) Describe the shape of the body as viewed from the side and front. ....

.... (b) How is the body form ideally suited for aquatic life? ....

.... Notice the movements of the mouth and operculum, or gill cover. (c) Describe the position of the operculum when the mouth is open. ....

.... (d) when the mouth is closed. ....

(e) Of what importance are the mouth and operculum movements in respiration? ....

....

Examine the eyes. (f) Is the eye protected by eyelids? .... (g) To what extent are the eyes movable in their sockets? ....

(h) Estimate the size of the pupil in proportion to the exposed area of the eye. ....

.... (i) Why is a large pupil an important adaptation of the fish?

....

The fins of a fish function in a variety of ways in locomotion, including rapid movement forward, slow movement forward, swimming backward, turning, maintaining a stationary position, balancing, and stabilizing. Examine the fins closely and determine their structure, number, location, and function. Summarize your observations in the following table.

Observe the fish closely as it is swimming forward rapidly. (j) Is most of the thrust provided by the caudal fin, or by the tail region of the body? ....

(k) Explain. ....

Name of fin	*Number*	*Location*	*Function*
Pectoral			
Pelvic			
Dorsal			
Anal			
Caudal			

A Part 2 EXTERNAL STRUCTURE OF THE YELLOW PERCH. The use of a preserved specimen of a bony fish will permit you to study its external structures more closely. As you examine your fish, notice how each part is adapted to aquatic life.

Materials. Fresh or preserved yellow perch (black sea bass or other carnivorous species may be used); compound or stereoscopic microscope; probe; forceps; dissecting tray; slide; cover glass.

Procedure and observations. Lay the fish in a dissecting tray with its head on your left. Open the mouth and notice the action of the upper and lower jaws. The upper jaw is the *maxilla.* The lower jaw is the *mandible.* Examine the maxilla and mandible for the presence of *teeth.* (a) Are teeth present in both jaws? .... Examine the roof of the mouth. (b) Are teeth present? .... (c) Describe the teeth of your fish. ....

(d) For what use are the teeth of the yellow perch adapted? ....

.... (e) Relate tooth structure to the diet and feeding habits of the yellow perch. ....

Examine the *tongue.* (f) Describe its structure and location. ....

....

(g) On the basis of structure and attachment of the tongue, determine its probable use. ....

.... (h) Describe the structure and location of the nostrils. ....

Insert your probe into one of the *nostrils.* (i) Does the probe enter the mouth cavity? ....

(j) Are the nostrils involved in respiration in the fish? .... (k) What is their probable use?

....

Probe into the throat cavity, or *pharynx.* (l) How many *gill arches* lie on each side of the pharynx? .... Insert your probe into the *gullet opening.* (m) Account for the large size and elasticity of the gullet opening of the yellow perch. ....

....

Press your probe against the eye. (n) To what extent is it movable? ....

.... Raise the *operculum* and examine the gills. Locate the membranous flap extending from the edge of the operculum. (o) Determine the function of this membrane by raising and lowering the operculum. ....

.... Examine the attachment of the ventral side of the head to the trunk. The narrowed extension of the body at this point is called the *isthmus*. (p) How is this modification in body structure important in the function of the gills? ....

....

Using your probe, raise the dorsal fin by pulling it forward. Notice that the dorsal fin of the yellow perch consists of a spiny portion and a soft portion. (q) Are the spiny and soft portions separate or attached in the yellow perch? .... (r) Count the number of spines in the spiny dorsal fin of your specimen. .... Compare the number of spines in your specimen with those of other members of the class. (s) Is the number constant or variable and, if variable, to what extent? .... (t) Describe the structure of the rays in the soft dorsal fin. .... (u) Examine all other fins of your specimen and determine if they are supported by spines, spines and rays, or rays: pectoral. .... ; pelvic. ....;
anal. ....; caudal. ....

(v) Locate and describe the *lateral line*. ....

.... The lateral line is believed to be a pressure organ. (w) How are the scales along the line adapted for exposure of sensory nerve endings? ....

....

Draw a lateral view of the yellow perch in the space provided and label: **maxilla, mandible, nostril, eye, operculum, scales, lateral line, spiny** (anterior) **dorsal fin, soft** (posterior) **dorsal fin, pectoral fin, pelvic fin, anal fin, caudal fin.**

Lateral view of the yellow perch

Examine the scales on your specimen. (x) Describe the manner in which they overlap. .... (y) Are the rows of scales vertical or oblique? .... Using your forceps, remove several scales in a small area. Probe into the pouches of the dermis in which the scales developed. Rinse a scale in water and mount it on a microscope slide with the outer surface up. Add a cover glass to keep the scale as flat as possible. Examine with the low power of your microscope or with a stereoscopic microscope. Notice the toothed edge of the posterior portion of the scale extending beyond the scale that overlapped it. As a fish grows, the size rather than the number of scales increases. As the scale grow, concentric rings are formed. Rings formed in the fall and winter are closer together than those formed during the spring and summer. By counting the number of regions of closely spaced concentric rings, you may determine the number of winters your specimen lived. (z)

What is the approximate age of your fish? ....

....

Draw an enlarged scale in the space provided and label: toothed edge and concentric rings.

C Part 3 EFFECT OF ENVIRONMENT ON COLOR CHANGES IN FISH

Problem. To what extent does the color of the environment alter the color pattern of a fish?

Protective coloration is important to survival of a fish. The colors of fish, like those of many other animals, serve to conceal them from their prey as well as from natural enemies. Many fish are concealed by simple countershading, in which darker colors above and lighter colors on the lower side produce a uniform coloration in the presence of bright light from the surface and reduced light in the deeper water. Other forms of protective coloration appear as bars, stripes, blotches, and spots which blend with water plants, rocks, and other objects in an aquatic environment. You can perform some experiments to determine whether environmental conditions cause variations in coloration of fish.

Fish scale (L.P.)

Suggested materials. Two sets of fish (3-5 in. long), one with scales and bars or spots (green sunfish are good) and the other lacking scales and with a more or less solid color pattern (bullhead catfish are good); aquarium for each fish; colored paper to cover the sides and bottom of the aquaria (white, brown, black, yellow, green, red); food for the fish (will depend on the species used).

Suggested procedure. Put each fish in an individual aquarium or battery jar with the water level slightly above the dorsal fin of the fish. The shallow water causes the fish to aerate the water by its own movement. Wrap the aquaria in each set of fish with paper of different colors, using white, brown, black, yellow, green, and red if possible. Be sure that the sides and bottom are entirely covered. Put the aquaria on a table so that the top light is the same for all of the fish. It will probably be necessary to change the water in all of the aquaria many times in the course of the experiment. Avoid exposing the fish to daylight when this is done.

Prepare a table for recording observations, listing the kind of fish in each set, and the color of the environment. Examine the fish each day, noting any color change. Continue daily observations for three to four weeks, or longer if desirable. Small fish (1-2 in.) may show noticeable color changes in a few days. Larger fish may require from several weeks to a month.

Analysis and conclusions. Several factors should be included in the report and analysis of your observations. To what extent did each fish change in coloration to a color resembling the background? Did some colors produce more change than others? If fish such as green sunfish, with variable coloration such as bars or blotches, were used, did color changes occur more in certain areas than in others? Did the two species used differ in their reaction?

Color change in fish involves pigment bodies in the dermis known as chromatophores. Use your text and other appropriate references to determine the structure and functioning of these chromatophores and how they are controlled by the nervous system. You should also determine whether any other factors are involved in color change in fish.

You may wish to carry your experiment further by sacrificing certain of your fish and preparing microscope slides of the dermis and examining the chromatophores for the amount of pigments present and dispersion or contraction of pigments.

Dissection of the Fish

34-2

Purpose To dissect a fish and study its internal anatomy.

In examining the internal structures of the fish, you will find that its organ-systems are more highly developed than those of the invertebrates you have studied previously. You will also become more familiar with the gill, the structure that enables the fish to respire in water.

A Part 1 STRUCTURE OF THE GILLS. The gills of the fish are bathed constantly by water. This enables the animal to obtain its necessary oxygen and release the carbon dioxide produced as a waste material.

Materials. Preserved fish (yellow perch is good); dissecting pan; dissecting scissors; forceps; scalpel; probe or dissecting needles.

Fish gill

Procedure and observations. Put the fish in the dissecting pan with its head to your left. Raise the operculum (gill cover) and examine the gills underneath. Insert your probe or the handle of your scalpel between the gills and look into the mouth cavity to see where it enters. Cut the operculum away with your scissors and remove one of the gills by cutting the upper and lower attachments of the *arch.* Rinse off the gill and examine it closely. Examine the feathery *filaments* and the comblike *rakers.* The arch is somewhat C-shaped and supports the gill. Examine the lower and upper ends of the arch and see if you can find the blood vessels that enter and leave the gill. Draw a gill in the space provided. Label: gill arch, filaments, rakers.

A Part 2 INTERNAL STRUCTURE OF THE FISH. The organs of the digestive system, reproductive system, and urogenital system are located in the body cavity of the trunk of the fish. The heart of the fish is literally "in its mouth," and the brain is elongated and composed of five parts.

Materials. Same as for Part 1.

Procedure and observations. Read the following directions carefully before starting. Each of the main organs you are to examine is indicated in *italics* in the directions. Hold the fish with the ventral side up and the head pointing away from you. Insert the point of your scissors through the body wall in front of the anal opening and cut up the midline of the body to the space between the gill covers on the lower side of the head. Lay the fish on its right side (with the head on your left) in the dissecting pan. Continue the incision from the isthmus, around the back edge of the gill chamber, to the top of the body cavity. Make another incision from the starting point of the ventral incision close to the anal opening, upward along the posterior wall of the body cavity to the top of the body cavity. Be careful not to disturb the organs underneath. With your scalpel make the remaining incision at the top of the body cavity and remove the side of the body wall. This procedure will expose the organs of the body cavity in their normal positions.

Locate the reddish-brown *liver* in the anterior end of the body cavity. Raise the lobes of the liver and find the *gall bladder* attached to the lower side. Cut the liver free where it is attached to the body and remove it. This will expose the short *esophagus* and *stomach.* Find the *pylorus,* where the stomach and *intestine* join. Locate the *pyloric caeca.* Examine the loops of intestine and find where it leads to the *anal opening.* Long masses of fat often lie along the loops of intestine. Cut the esophagus where it leads from the mouth and carefully remove the entire alimentary canal, from the mouth to the anal opening. Draw the alimentary canal in the space provided and label: **esophagus, stomach, pylorus, pyloric caeca, intestine.**

With the alimentary canal removed, you should see the gonads clearly and perhaps the *urinary bladder.* The female will have a large, yellowish *ovary* containing numerous eggs. The male will have smaller, white *testes.* Trace the white, threadlike ducts from these organs and the small tube from the urinary bladder to the *urogenital opening* posterior to the anal opening.

Alimentary canal of a fish

Locate the *air bladder* along the top of the body cavity. The air bladder may have been broken in removing the body wall. Find the *kidneys,* which appear as dark red masses along the spine, above the air bladder.

Section the isthmus carefully, and open the *pericardial cavity,* containing the *heart.* Find the *cardinal vein,* the large vein which carries blood from the body into the heart. This empties into a thin-walled sac, the *sinus venosus,* on top of the heart. The upper chamber (posterior) is the *atrium.* Below the anterior to the atrium is the muscular *ventricle.* A muscular bulb, the *bulbus arteriosus,* joins the ventricle and gives rise to the *ventral aorta,* which branches to the gills. Remove the heart, and draw it in the space provided. Label: **cardinal vein, sinus venosus, atrium, ventricle, bulbus arteriosus, ventral aorta.**

Fish heart

Now hold the fish with the dorsal side up and the head pointing away from you. Using your scalpel, cut the skin from the skull. Scrape the skull carefully to wear away the bone. When the bone is very thin, pick out the pieces with your forceps to expose the brain. Locate the *olfactory lobes* in front, the larger lobes of the *cerebrum* behind these, and the very large *optic lobes* posterior to the cerebrum. The *cerebellum* is posterior to the optic lobes, and the *medulla* is the enlargement where the *spinal cord* joins the brain. Draw the brain in the space provided. Label the **brain regions** and the **spinal cord.**

Fish brain

Effect of Temperature on Goldfish Respiration Rate

34-3

Purpose To determine the influence of water temperature on the respiration rate of goldfish.

Cold-blooded animals are literally at the mercy of their environment. Lowering of the body temperature in response to reduced temperature of the environment may result in the crystallization of proteins in the blood, resulting in death. An increase in body temperature may speed up metabolism to such an extent that the tissues are literally burned up.

B Part 1 EFFECT OF WATER TEMPERATURE ON THE RESPIRATION RATE OF THE GOLDFISH. The respiratory cycle in the fish is accomplished when the water, laden with oxygen, enters the mouth and is forced out over the gill filaments when the mouth is closed. The oxygen dissolved in the water enters the capillaries, and the excess carbon dioxide in the capillaries is released into the water. The operculum opens to allow the carbon-dioxide laden water to leave the gill chamber, thus completing the respiratory cycle. Therefore, the counting of the movements of the operculum is one method of computing the respiratory rate of the goldfish.

Materials. Aquaria or battery jars; goldfish (comets are best for this experiment); thermometer that can be immersed in water; crushed ice; timing device; warm water.

Procedure and observations. Put a goldfish in an aquarium or battery jar with sufficient water to cover the dorsal fin. Put a thermometer at one edge of the aquarium so you can read it without disturbing the fish.

In order to reduce the shock factor and avoid exciting the fish, add crushed ice slowly to the aquarium. Continue to add the ice until the water temperature is reduced to near freezing.

Now observe the movement of the operculum covering the gills. Use your timing device and count the movements of the operculum at the lowest temperature for one minute. Record this rate in the table provided.

Slowly add warm water to the aquarium at the end opposite the thermometer. Remove some of the water and ice so that the water volume remains constant. *Avoid exciting the fish* when you are adding the water. (a) Explain why this is necessary. ....

.... Continue to add warm water until a five-degree rise in temperature occurs. Wait a few seconds for the fish to adjust to the change, then count and record the rate of operculum movement at this temperature. Continue adding warm water to the aquarium, remembering that some water will have to be removed, and record the rate at each five-degree temperature interval until the water temperature reaches 92°F. If you use a centigrade thermometer, convert this temperature.

After the experiment remove the goldfish and place it in an aquarium at room temperature and well aerated. Calculate the average rate of operculum movement for the goldfish, record in the table provided, and construct a graph based on the averages.

(b) Discuss your observations of the fish during this experiment. ....

....

....

(c) At which temperature did the greatest number of operculum movements occur? ....

Degrees Fahrenheit	*Fish #1*	*Fish #2*	*Fish #3*	*Fish #4*	*Average rate*
32					
37					
42					
47					
52					
57					
62					
67					
72					
77					
82					
87					
92					

Interpretation. What is the relationship between the increase in water temperature and respiration rate in the goldfish? ....

List the factors which, in addition to water temperature, could influence the respiration rates in your goldfish. ....

....

Explore other factors such as the effect of heat or extremely cold temperatures on the enzymes involved in respiration, or the effect of oxygen starvation on internal tissues of the fish. ....

....

....

Fish are adapted for an aquatic life and live in both warm and cold waters. In terms of your results, discuss how the respiration rates of fish in these environments could vary. ....

....

....

External Structure of the Frog 35-1

Purpose To study the external structure and characteristics of a tadpole and adult frog, and to study regeneration in amphibians.

In your study of the frog, you will become familiar with one representative of a group of animals that, while they represent the first land-dwelling vertebrates, have never left the water entirely. Although they have lungs, their thin skin is the most important organ of respiration. Thus, it must remain moist at all times. The webbing on the hind toes is more beneficial to a water-dwelling organism than to a terrestrial one. Furthermore, the frog and its relatives must return to the water to lay their eggs, and the tadpole remains aquatic until it develops lungs and legs and absorbs its caudal fin.

A Part 1 EXTERNAL STRUCTURE OF A TADPOLE. The metamorphosis of the frog illustrates an amazing development from an aquatic, fishlike animal, the tadpole, to a semiaquatic, adult amphibian, the mature frog. The bullfrog tadpole is an excellent subject to use because it is large and because it spends two winters and two to three years in its various tadpole stages. Thus, at any given time, you can find bullfrog tadpoles in various stages of development.

Materials. Living bullfrog tadpoles in various stages of development; aquaria to house the tadpoles; charts showing metamorphosis.

Procedure and observations. Study the various stages of development in the living tadpoles or use charts showing the metamorphosis. Notice the well-developed *tail,* surrounded by the *caudal fin* on many of the bullfrogs. The tail and fin are richly supplied with blood vessels. Notice that the caudal fin and tail are not so prominent on some of the bullfrogs. (a) What has happened to the tail and caudal fin on these animals? ....

....

Examine the bullfrogs for evidence of legs. (b) Which legs appear first? .... Observe some of the bullfrogs that have *hind legs.* (c) Are they of any use to the tadpole? .... Examine the mouth and *horny lip.* (d) Describe the shape of the mouth. ....
(e) How is the horny lip used in feeding?

....

Examine the mouth of bullfrog tadpoles that have their hind legs. (f) How does the mouth differ from that of a tadpole lacking legs? ....

Young tadpole without legs

Older tadpole with hind legs

Notice the eyes on the tadpoles that lack legs; then examine the eyes of the ones with hind legs. (g) How do they differ? ....

.... Gills are situated on either side of the head and are covered by a fleshy *operculum.* Locate the *spiracle* on the left side. This is the opening into the gill chamber. Notice that water enters the mouth and is forced out through the spiracle as in the fish. Examine the tadpoles for buds under the operculum from which the fore legs develop. This is a critical period in the life of the tadpole because the left fore leg closes the spiracle opening. If the fore leg emerges before lungs develop, the animal may die. (h) What other amphibian does the tadpole resemble in later stages, when it has four legs and a tail? ....

Draw a young tadpole in the space provided and label: **spiracle, horny lip, operculum, tail,** and **caudal fin.** Draw in the space provided an older tadpole whose hind legs have emerged and label: **eye, mouth, tail, caudal fin,** and **hind leg.**

A Part 2 EXTERNAL STRUCTURE OF THE FROG. In your study of the external structure of the frog, you will become familiar with the various structures that adapt the frog to its amphibious life.

Materials. Living frog; preserved frog; microscope; battery jar or aquarium; dissecting tray; probe; cover glasses; slides; scissors.

Procedure and observations. Put a live frog in a battery jar or aquarium with sufficient water for the animal to swim. Touch the frog with your probe and note how it swims. (a) Describe the swimming movements. ....

....

Study the frog's eyes. (b) What color is the iris? .... (c) Are eyelids present? .... (d) Can the frog move its eyes? .... Put the frog on a table or on the floor. Touch the animal with your probe to get it to jump. (e) Describe what happens. ....

....

Leave the frog out of the water and watch the breathing movements it makes. (f) Record the action of the floor of the mouth and the muscles of the thoracic cavity. ....

....

Now examine a preserved specimen for other structures. The *nostrils* are located just in front of the eyes. (g) How many nostrils does the frog have? .... Introduce a probe into the nostrils. (h) Do they enter the mouth cavity? .... (i) Explain your answer. .... Examine the thin *nictitating membrane* that covers the eye from below. (j) Of what advantage would this membrane be to the frog while under water? ....

.... (k) on land? ....

.... Locate the large *tympanic membranes* (eardrum) posterior to the eyes. Notice that the mouth actually extends from ear to ear, forming a perfect insect trap. (l) What is the general shape of the body? .... Examine the fore legs. (m) How many fingers are present? .... (n) Are they webbed? .... Stretch out the hind legs. (o) What is their approximate length in proportion to the body? .... (p) How many toes are present? .... (q) Are they webbed? .... Locate the *anus* at the posterior end of the body. Draw a dorsal view of the frog in the space provided and label: **mouth, eye, nictitating membrane, nostril, tympanic membrane, fore leg, hind leg, webbing, anus.**

Dorsal view of the frog

Note the arrangement of the spots and the coloration of the frog. The color of the frog is due to a set of structures called *chromatophores.* These pigment cells are located in the dermal layer of the skin. They are usually star-shaped with rays extending from the central body. The pigment in the chromatophores is in the form of tiny granules which may be dispersed into the rays or concentrated in the central body of the chromatophores. Dispersal of the pigment causes the frog to darken in color; contraction of the pigment into the center of the chromatophores results in a lighter color. The presence of many chromatophores in the skin results in the characteristic spots of the leopard frog.

Remove a section of the dorsal skin containing one of the frog's spots, and mount it on a slide in a drop of water, using a cover glass. Examine the entire section under low and high power of your microscope. (r) Is the dispersal of pigment uniform in all the chromatophores you observed? .... (s) How is the dispersal of pigment related to the color of the spot and its boundary? ....

Now remove a second section from a lighter area of the dorsal skin and examine under the microscope. (t) How do the chromatophores of this section differ from those of the darker area? .... With your fine adjustment, focus below the dermal layer of skin and look for the irregularly shaped silvery white structures located there. These are *guanin crystals,* which are responsible for iridescence. They are usually overshadowed by the concentration of chromatophores in the dermis of the dark areas. (u) How numerous are the guanin crystals on your side? ....

Examine a section of skin from the ventral surface. (v) What is the relationship between the color and the presence or absence of chromatophores or guanin crystals? ....

....

Draw several chromatophores and a guanin crystal in the space provided. Identify the area of skin from which the drawing is taken.

Chromatophores

Guanin crystal

C Part 3 REGENERATION IN A TADPOLE

Problem. Does a tadpole have the capacity to regenerate a tail fin?

Many living things have the ability to regenerate lost parts. The hydra can grow into a complete animal from a very small section of its body tissue. The planarian can regenerate an anterior and posterior end from certain sections of its body. The earthworm can regenerate a head. These are all invertebrate animals and less specialized than a tadpole, which is a vertebrate. You can determine whether a vertebrate animal can regenerate lost parts by cutting off a portion of its tail fin.

Suggested materials. Tadpoles of the leopard frog in various stages of growth; scissors; razor blade; Petri dishes; culture dishes; narcotic solution (MS 222 1:2500 or chloretone 1:2500).

Suggested procedure. Transfer the leopard frog tadpoles to Petri dishes containing the narcotic solution. Wait until the animals are motionless. Then, using a razor blade or scissors, cut carefully across the tail fin at an oblique angle about 4 mm from the tip. Put this animal in a culture dish of water. Operate on several of the tadpoles, changing the angle of the cut.

Feed the animals spinach or egg yolk every other day. *Change the water daily to avoid pollution by bacteria, which can harm the tadpoles.* Watch the animals carefully and note when the wound is healed, when regeneration begins, when the regenerated portion becomes pigmented, and whether the angle of the cut influences regeneration in any way. Record any unusual development in the tail fin.

Analysis and conclusions. Your data may be recorded as sketches made following the removal of the tail fins and each day thereafter.

Dissection of the Frog

Purpose To dissect a frog and study its internal structure.

The dissection of the frog is a study of the anatomy of the vertebrate body as well as a valuable introduction to human biology.

A Part 1 STRUCTURE OF THE MOUTH. As you study the structures of the mouth, you will become familiar with the adaptations that enable the frog to obtain food, particularly insects.

Before starting your dissection, read the directions carefully and make sure that you understand all parts of the procedure. Organs to be located will be mentioned in *italics*. You may want to check their location in the "Trans-Vision" of the frog in MODERN BIOLOGY.

Materials. Preserved frog; dissecting pan; dissecting scissors.

Procedure and observations. Lay the preserved frog on its back. Cut the jaws apart at the hinge in order to open the mouth wide. Locate the *tongue* in the floor of the mouth. (a) Where is it attached? .... Feel the lower surface of the tongue. (b) Why is the tongue sticky in the living frog? ....

(c) Explain why the tongue is forked. ....

If you have a male frog, find the openings to the *vocal sacs,* near the hinge on the lower jaw on either side. The openings lie far back in the center of the mouth. The lower one is the slit-like *glottis.* (d) Where does it lead? .... The upper one is the *gullet opening.* (e) Where does this opening lead? ....

The openings of the *Eustachian tubes* are in the roof of the mouth, near the hinge of the upper jaw. (f) To what organs do the Eustachian tubes lead? ....

....

Locate the *nostril openings* in the roof of the mouth near the tip of the head. Between the nostril openings are the two *vomerine teeth* which project from the roof of the mouth. (g) What is their function? ....

Examine the jaws of the frog. (h) Which of the jaws is provided with small teeth? ....

Locate the lower sides of the *eye sockets.* (i) Of what advantage are these eye sockets? ....

....

A Part 2 DISSECTION OF THE BODY CAVITY. Because of similarities, the study of the organ-systems of the frog will help you to understand human physiology and anatomy.

Materials. Preserved frog; hand lens or stereoscopic microscope; dissecting pan; dissecting scissors; scalpel; forceps; probe.

Procedure and observations. Insert the point of your scissors through the skin just above the anal opening and make an incision extending to the lower jaw. Cut around the edge of the body on both sides and remove the pieces of skin. Examine the inner side of the skin and note the pattern of the blood vessels. Study the muscle layers exposed by the removal of the skin. Now remove the muscle layers in the same manner in which you removed the skin. In the region of the thorax it will be necessary to cut through the bones of the arms and the shoulder. Remove the muscle layers with your forceps.

If your specimen happens to be a female, the body cavity may be nearly filled with eggs. If so, it will be necessary to remove the eggs with your forceps before proceeding further. Note that the eggs are loose in the body cavity. You will also find two long, coiled, white *oviducts* leading from the anterior end of the body cavity to the posterior end. These are the tubes through which the eggs pass and should not be confused with other tubular structures to be referred to later.

Find the large three-lobed *liver* high in the body cavity. Spread the lobes of the liver apart with your probe and find the bright green *gall bladder.* Raise the liver on the left side and notice the position of the *stomach.* Follow the *intestine* from the lower end of the stomach to the *large intestine.* Examine the *mesentery* and the manner in which it holds the intestine in position. Find the *spleen* on the mesentery. The *pancreas* is attached to the lower part of the stomach.

Alimentary canal of frog

Next remove the liver, using care not to damage other organs. Cut through the *gullet* at its upper end. Raise the stomach with your forceps and carefully loosen the intestine from the mesentery with your scissors. Cut through the large intestine as far down as possible. The *cloaca* lies below the large intestine, between the hind legs. Lay the alimentary canal in your dissecting pan. Pin the stomach at the top and the large intestine at the bottom. Find the muscular valve, or *pylorus,* at the lower end of the stomach, where it joins the intestine. Draw the alimentary canal in the space provided. Label: **gullet, stomach, pylorus, intestine, large intestine.**

Using your scissors and beginning at the pylorus, cut along the outer curvature of the stomach and gullet. Pin the flaps back and examine the contents, if any, and the lining of the stomach. (a) Describe this lining. ....

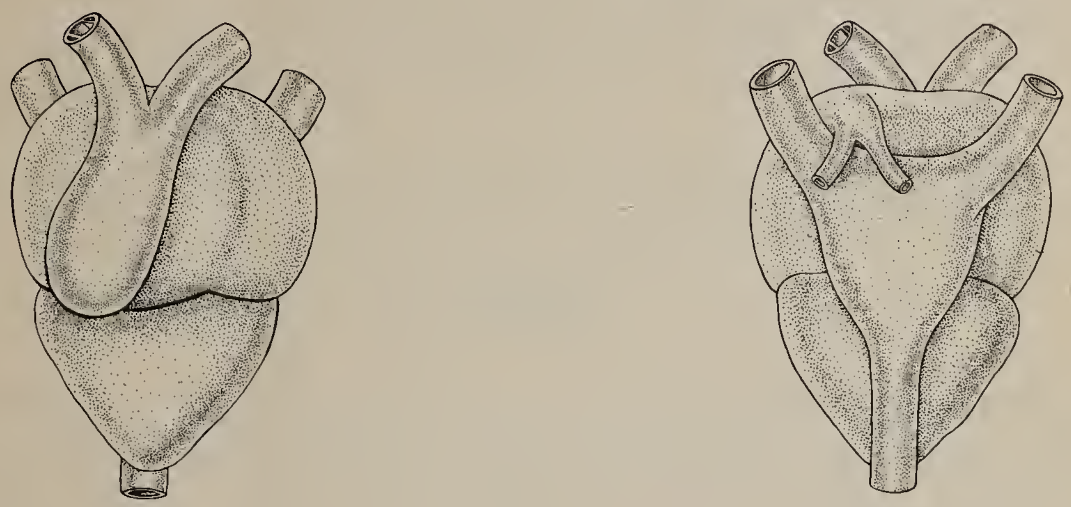
(b) Account for its structure. ....
Now cut through the pylorus and continue the cut along the length of the small intestine. Examine the lining of the small intestine. (c) Compare it with the lining of the stomach.

.... Now use a hand lens or stereoscopic microscope to examine the lining. (d) Describe what you find. ....

.... (e) Explain the function of these projections.

....

Examine the *heart,* which lies in a thin sac, the *pericardium.* Using your probe, find the great veins and arteries leading to and from the heart. Examine the *lungs* and notice the pulmonary veins and arteries connecting the heart and lungs. See if you can find the *trachea,* leading from the mouth to the lungs. Remove the heart, severing the necessary blood vessels. Examine the front side of the heart with your hand lens. The large vessel arising from the ventricle, extending over the front surface of the heart and forming a Y at the top of the heart, is the *conus arteriosus.* This great compound vessel sends one branch to the head, a second to the body, and a third to the lungs and skin. Locate the *right atrium, left atrium,* and *ventricle* in the front view of the heart. Examine the back side of the heart with your hand lens. Three branches of the *vena cava* unite in a thin-walled sac, the *sinus venosus,* on the back wall of heart. The sinus venosus opens into the right atrium. *Pulmonary veins* from the lungs join the left atrium. Examine the front and back views of the heart shown in Fig. 35-2-a. Label: **ventricle, right atrium, conus arteriosus** in the front view and **ventricle, right atrium, left atrium, vena cava, sinus venosus, pulmonary veins** in the back view.



Front view

Back view

Fig. 35-2-a Frog heart

Examine the dark red, lobed *kidneys* lying along the back on either side of the spine. Find a small, twisting tube, the *ureter,* leading from each kidney to the cloaca. The urinary bladder attaches to the cloaca and receives urine from the cloaca for storage. Do not confuse the ureters with the oviducts in a female. If your frog is an immature female, you will find two-lobed, grayish *ovaries* lying close to the kidneys. The *testes* of the male are in a corresponding position. Find the yellow, fingerlike *fat bodies* attached above the kidneys.

A Part 3 DISSECTION OF THE BRAIN. In your study of the frog's brain you will recognize that it is similar to that of the fish, but that in certain respects it is more highly developed.

Materials. Same as for Part 2.

Procedure and observations. Turn the frog over so the dorsal side is up. Insert the point of your scissors through the skin at the base of the head and make an incision forward to the tip of the jaw. Remove the skin from this area. With your scalpel or scissors, cut through the top of the skull. Use extreme care not to cut too deeply. Spread the bone apart to expose the brain. Find the **olfactory lobes, cerebrum, optic lobes, cerebellum,** and **medulla oblongata** from front to back. Draw the brain in the space provided. Label these brain regions and the end of the **spinal cord.**

The frog is considered a more advanced vertebrate than the fish. From the knowledge of five systems you studied in considerable detail, indicate the extent to which this is true, based upon the development of these five systems.

Frog brain

1. Digestive system

2. Respiratory system

3. Circulatory system

4. Nervous system

5. Excretory system

Effect of Drugs and Hormones on the Metamorphosis of Amphibians

35-3

Purpose To study the effect of drugs and thyroid hormones on the metamorphosis of amphibians.

Metamorphosis in amphibians consists of many internal and external changes. The most conspicuous in the frog are the loss of gills, absorption of the tail and caudal fin, emergence of legs, bulging of the eyelids and formation of eyelids, change in body shape, and altering of pigmentation.

Growth and differentiation in body tissues are controlled by hormones secreted by glands. Metamorphosis in frogs is an example of this control. Thyroxine, produced by the thyroid gland, plays an important role in metamorphosis. If the animal does not obtain sufficient iodine to form thyroxine, the larva fails to metamorphose.

B Part 1 STUDY OF EFFECTS OF DRUGS AND HORMONES ON FROG METAMORPHOSIS. In this Part you will use iodine and thyroxine as contained in thyroid tablets to determine their effects on metamorphosis in the frog tadpole. You will administer propylthiouracil, which is a thyroid inhibitor, to determine its effects.

Materials. Twenty-four bullfrog tadpoles; refrigerator; small aquaria or battery jars to house the tadpoles; Petri dish; glass-marking pencil or gummed lables; analytical balance; mortar and pestle; glass plate 4 × 4 in.; wide-mouth jar for thyroid wheat mixture; two 1,000-ml ground-glass stoppered tincture bottles; 100-ml graduate; 1-ml pipettes; 1-liter volumetric flask or graduate; spring or pond water (600 ml per jar or aquarium); whole wheat flour; strained spinach or hard-boiled egg yolk; 1-mm graph paper; 2-grain thyroid tablets; 50-mgm propylthiouracil tablets; 5 gm crystalline iodine; 10 gm potassium iodide crystals; distilled water.

Procedure and observations. Before you begin your study of the metamorphosis of the frog, prepare a stock solution of thyroid-wheat mixture by weighing on an analytical balance an amount of whole wheat flour equal to the weight of five 2-grain thyroid tablets. Grind the tablets and flour together in a mortar. Add 5 ml of distilled water to the ground mixture and spread the resulting paste on a clean glass plate. Allow the paste to dry. Then grind the dried mixture and put it in a stoppered, labeled bottle and refrigerate until needed.

Prepare an iodine stock solution by placing 5 gm of crystalline iodine and 10 gm of potassium iodide in a 1,000-ml volumetric flask or graduate and add 100 ml of distilled water. Then add sufficient distilled water to make 1 liter. Refrigerate until needed.

Prepare a propylthiouracil stock solution by dissolving one 50-mgm propylthiouracil tablet in 100 ml of distilled water and add distilled water to make 1,000 ml of solution. Put in a stoppered bottle, label, and refrigerate until needed.

Since some of the tadpoles may die, use at least 18 for this study. Put three tadpoles into each aquarium containing 600 ml of spring or pond water. Culture dishes with less water may be used if the tadpoles are small. You may use tap water in the aquaria if it has been conditioned by aeration for one-half hour or by allowing the water to stand for two weeks in an open container.

Number the aquaria 1-6. To the water of aquarium 1, add 0.05 gm of the thyroid-wheat flour stock mixture daily. Add 2 ml of the iodine stock solution to aquarium 2 daily. To aquarium 3, add 1 ml of the iodine stock solution daily. Add 0.5 ml of iodine stock solution to aquarium 4 daily. Add 1 ml of the propylthiouracil stock solution to aquarium 5 daily. The tadpoles in aquarium 6 are to serve as controls.

The cultures should be kept at room temperature (70°F). If the temperature varies slightly, it should not harm your animals, but extreme variations will kill them. All animals, experimental and controls, should be fed a diet of spinach or hard-boiled egg yolk every other day. *Change the water daily to avoid pollution by the overproduction of bacteria, which can harm the animals.*

Observe and measure the animals at four-day intervals for six weeks (if you use smaller tadpoles, observe for two weeks). When you are ready to measure an animal, put it in a Petri dish to which you have taped a piece of millimeter graph paper. Make and record measurements of the total length of the animal, the length of the body from snout to the base of the tail, the width of the body at the snout, and the width of the body at the base of the tail. Prepare a graph plotting the mean length and width of all measurements of each group every four days during the period of your observations.

(a) What is the first significant change in the animals? ....

.... (b) In which group did it occur?

.... (c) Discuss the changes and

the sequence in which they occurred. ....

....

....

.... (d) Was the sequence of change the

same for all animals? .... (e) If not, list

as many reasons as you can to account for the difference. ....

....

....

.... (f) Did any of the animals die before

the experiment was completed? .... (g) If so, cite as many reasons

as you can to explain their death. ....

.... (h) Which animals, if any,

metamorphosed first? .... (i) Explain

your answer. .... Iodine is not

a thyroid hormone. (j) Explain why it is of value in the study of metamorphosis. ....

....

....

(k) How does propylthiouracil affect the tadpoles? ....

.... (l) Describe the propylthiouracil-

treated animals at the end of the experiment. ....

....

....

Interpretation. On the basis of your experiment, explain how you have demonstrated that metamorphosis is related to the activities of the thyroid gland. ....

....

....

....

B Part 2 EFFECT OF THYROID HORMONES ON THE METAMORPHOSIS OF THE MEXICAN AXOLOTL. The Mexican axolotl is an amphibian that lives in cold lakes and streams lacking in iodine. Since thyroxine, which controls metamorphosis, is not produced without iodine, these animals remain in the larval stage. However, axolotl larvae reach sexual maturity and can breed. In this Part you will determine whether metamorphosis can be induced in the axolotl.

Materials. Four Mexican axolotls (3-5 inches in length); refrigerator; four aquaria; 1-ml pipette; scalpel; forceps; 2 stoppered bottles; beef thyroid gland tissue; frozen liver; analytical balance; metric ruler; thyroxine crystals; triiodothyronine crystals; distilled water.

Procedure and observations. Prepare a stock solution of thyroxine by dissolving 0.25 gm of the thyroxine crystals in 400 ml of distilled water. Put the solution in a stoppered bottle and refrigerate until needed.

Prepare a stock solution of the triiodothyronine by dissolving 0.25 gm of the triiodothyronine crystals in 400 ml of distilled water. Put the solution in a stoppered bottle and refrigerate until needed.

Number the aquaria *1-4* and put 600 ml of water in each. Put an axolotl in each aquarium.

(a) Describe the larval animals. ....

....

The animals should be fed frozen liver every other day. Take a small piece of the liver on a pair of forceps and hand feed the animals. Clean the aquaria after each feeding. Then into aquarium 1 put 1 ml of thyroxine on alternate days. Put 1 ml of triiodothyronine into aquarium 2 and feed the animal in aquarium 3 a piece of the thyroid gland tissue on alternate days. The axolotl in aquarium 4 will serve as a control.

Measurements should be made of the animals when you clean the aquaria. You can handle the animals and use a metric rule to measure the length of the gills, the length of the fin from the base of the tail to the tip, and the width of the tail. Prepare a graph of the measurements. A separate graph can be used for each measurement. Make a table for all measurements.

(b) Which animal showed the first evidence of metamorphosis? ....

.... (c) In what way did it show that metamorphosis was occurring?

.... (d) Which of the axolotls

completed metamorphosis first? ....

(e) Describe all of the visible changes that occurred in the animals. ....

....

.... (f) How does the control differ from the metamorphosed animal? ....

.... (g) Account for this difference.

....

Interpretation. On the basis of your observations, explain why the axolotl remains in a larval stage in its native habitat, but may undergo metamorphosis when it inhabits other streams.

....

....

....

Purpose To dissect a mammal and to study its internal organs and systems.

The fetal pig is an excellent and widely used subject for mammalian dissection. The organs are large and are, in many respects, similar to those of your own body.

B Part 1 DISSECTION OF THE FETAL PIG. Since your specimen is a fetal animal, you will find several structures characteristic of an unborn mammal. In its uterine development, the placental mammal is provided the greatest possible protection and a period of development sufficient to form the many specialized structures of this most highly developed organism.

Materials. Preserved fetal pig; dissecting pan; dissecting scissors; scalpel; forceps; probe or dissecting needles; bone cutters or heavy forceps or pliers.

Procedure and observations. Section 1 *Preliminary dissection.* Put the animal in the dissecting pan, ventral side up. Tie the legs securely to the corners of the pan, or by passing a string under the pan from front leg to front leg and hind leg to hind leg. Be sure that the specimen is held firmly before you begin dissecting.

Locate the *umbilical cord* and with a needle mark off a strip on the abdomen about one-fourth inch to either side and one inch anterior and posterior to the cord. With the scissors, make an incision on a midline between the hind limbs and cut forward and around the umbilical cord. Continue with a midline incision to the base of the *sternum.* Then make lateral incisions posterior to the forelimbs and anterior to the hind limbs.

Separate the layers of skin, pin them back and expose a thin membrane, the *peritoneum,* which lines the abdominal cavity. Cut through the peritoneum to expose the abdominal organs. Locate and cut the *umbilical vein,* which connects the umbilical cord with the liver. Now you can lay back the umbilical cord and its strip of skin. Locate the *allantoic duct, umbilical arteries,* and *umbilical vein* in the cord. Introduce the tip of a probe into each vessel and determine its destination. Under the strip of skin left around the umbilical cord locate the long narrow sac called the *allantoic bladder.* This is the fetal excretory organ.

Section 2 *Examination of the abdominal organs.* Locate the *diaphragm,* which separates the thoracic from the abdominal cavity. Identify the *liver,* which occupies the upper right area of the body cavity just below the diaphragm. Count the lobes. Raise the liver with your forceps and find the prominent *gall bladder.* Locate the various *hepatic ducts,* which lead from the liver to a branch, the *cystic duct,* which extends to the gall bladder. These ducts join to become the *common bile duct,* which empties into the *duodenum.* Notice the blood vessels which lead to the liver from the lower side and to the *heart* from the upper region. The *stomach* is very prominent, although it may be partially hidden by a lobe of the liver. Find the *gullet,* which passes through the diaphragm and enters the stomach at its upper end. At the point where the lower end of the stomach joins the small intestine is a muscular valve, the *pylorus.* The red, elongated organ extending around the stomach in the fetal pig is the *spleen.* Even though it is located close to the stomach and intestines, it is not a digestive organ. The *pancreas* is large in the mammal and is located below the stomach. It appears as a white, granular organ. Examine it closely to see if you can find the *pancreatic duct,* which empties into the duodenum near the entrance of the bile duct.

The *small intestine* is a coiled and twisted tube, many feet in length. It is held in place by fanlike folds of the *mesentery.* Raise the small intestine at several points and examine the mesentery. Note the many blood vessels along the surface of the intestine, through which absorption of food takes place. The small intestine begins at the stomach and ends at the junction with the large intestine. The *large intestine* appears as a compact coil. Find the junction of the small and large intestines. Below the junction is a large, blind end, the *caecum.* The caecum may be likened to the appendix in man. Trace the large intestine from the caecum to the point where it leads to the *anus.*

In Fig. 38-1-a label: **liver, stomach, spleen, pancreas, small intestine, large intestine, allantoic bladder, umbilical artery, umbilical vein.**

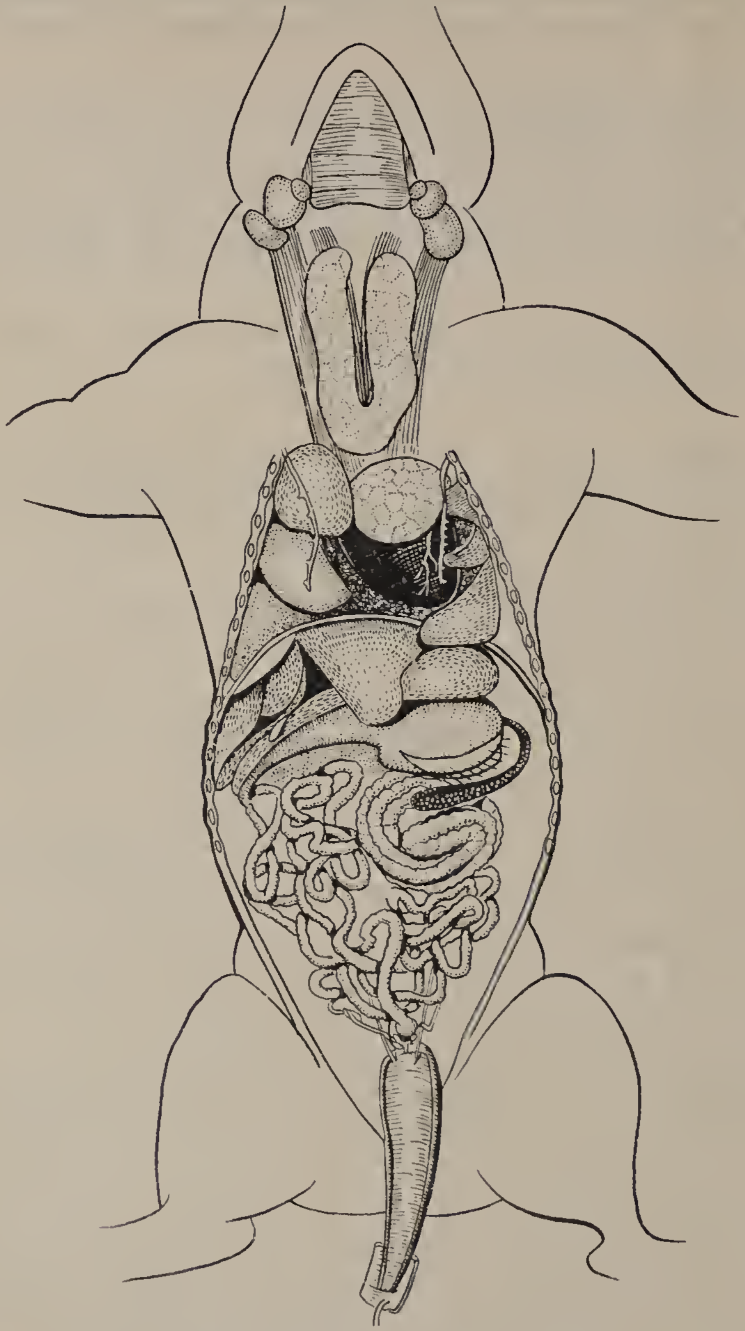


Fig. 38-1-a Internal organs of the fetal pig

If you want to study the abdominal organs more thoroughly, continue as directed. Cut the gullet, passing through the diaphragm. Using your scissors, cut the membranes which fasten the digestive organs along the back. Continue cutting membranes, using care *not* to cut the large blood vessels running along the back or to damage the kidneys and ureters. Follow the lower end of the large intestine and cut it just above the anal opening. You should now be able to lift out the digestive organs and the spleen. Unravel the small intestine from the stomach to the large intestine and measure its length. Locate the three regions of the small intestine—the *duodenum, jejunum,* and *ileum.* Loosen the coils of the large intestine and determine the length of this portion. Now estimate the length of the gullet you removed and, by adding the lengths of the gullet, stomach, small and large intestine, determine the total length of the alimentary canal. Section the stomach and pylorus laterally and examine the muscle layers. Examine the *cardiac sphincter* valve, *pyloric sphincter* valve, and *diverticulum* (a blind pouch of the upper region of the stomach). Section lengthwise the junction of the small and large intestine and locate the *ileocaecal sphincter* valve. Discard the digestive organs.

Section 3 *Examination of the thoracic organs.* Before cutting away any part of the diaphragm, locate the large blood vessel, the *postcaval vein,* which passes through the lobes of the liver into the heart. Clip the edges of the diaphragm and separate it from the body wall, being careful not to cut the postcaval vein. When the diaphragm is free, let it drop down over the liver. Make a mid-ventral incision through the entire length of the sternum from its lower end to the base of the neck.

Spread the ribs apart and locate the *heart.* It lies in a thin sac, the *pericardium,* and is partially covered by the *thymus gland.* On either side of the heart are the large, spongy *lungs.* Observe that the lungs are completely collapsed because the animal has never drawn a breath. Find the *trachea,* which leads to the lungs. Just above the lungs, it divides to form a Y. Try to find this with your probe. Immediately posterior to the trachea is the gullet, which leads to the stomach.

Continue the dissection into the neck following the trachea. Note that the thymus gland extends far into the neck. Probe between the lobes of the thymus gland carefully. At the anterior end of the thymus you should find a reddish, oval gland lying over the trachea just below the *larynx.* This is the *thyroid gland.* If you continue the dissection into the lower jaws and cheeks, it is possible to locate the large *salivary glands.*

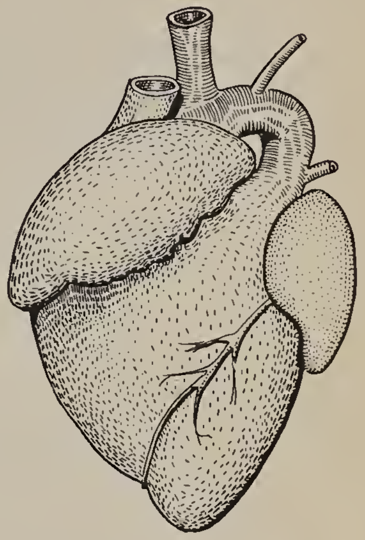


Fig. 38-1-b Ventral view of the pig's heart

In Fig. 38-1-a label: **heart, thymus gland, lungs, trachea, thyroid gland.**

Cut around the larynx, high in the neck, and raise it with your fingers. Using your scalpel, cut along the trachea, lifting it as you cut. You should be able to lift the lungs and heart from the specimen without difficulty. Cut the large blood vessels. Find the *aorta,* which passes between the lungs on the back side from the heart. Cut the aorta loose and push it to one side. The other tube is the gullet. Loosen the gullet, trace it to the upper side of the larynx, and remove it. Now find the *epiglottis,* a leaflike structure of fibro-cartilage at the top of the larynx. Notice the *cartilage rings* composing the trachea. Follow the trachea to the Y just above the lungs. Each of these divisions is called a *bronchus.*

Remove a bulb from a pipette and insert the smaller end through the epiglottis, blow on it and try to inflate the lungs.

Using your forceps, begin to remove the lung tissue to expose the "bronchial tree" (this may also be done by scraping carefully with a scalpel). After the lung tissue has been removed, float the bronchial tree in water and observe under a broad-field microscope. Try to find the *air sacs.*

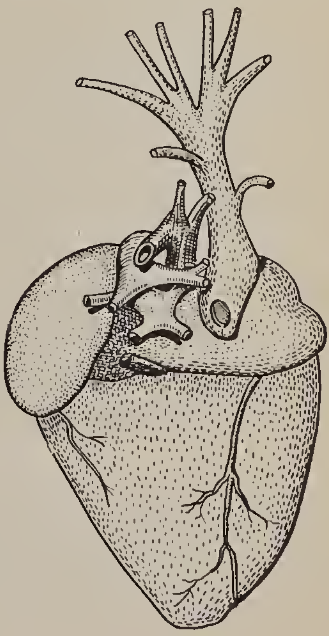


Fig. 38-1-c Dorsal view of pig's heart

Remove the heart from the lungs by cutting the *pulmonary arteries* and *veins*. If the heart is sufficiently large, you should find the *venae cavae* and *pulmonary veins* leading to the *atria* and the *aorta* and *pulmonary artery* leading from the *ventricles*. A characteristic feature of a fetal mammalian heart is a short artery connecting the pulmonary artery and aorta. This vessel, which allows blood to bypass pulmonary circulation until birth, is known as the *ductus arteriosus*. It is very prominent in the fetal pig. Section the heart laterally and examine the chambers. The stringlike structures in the ventricles are *tendons* controlling the action of *valves*. In Fig. 38-1-b label: **left atrium, left ventricle, right atrium, right ventricle, aorta,** and **pulmonary artery.** In Fig. 38-1-c label: **left atrium, pulmonary vein, left ventricle, vena cava, right atrium, right ventricle,** and **ductus arteriosus.**

Section 4 *Examination of the urogenital system.* The urinary system is composed of the *kidneys, bladder,* and *ducts.* You have already located the allantoic bladder and duct. Locate the kidneys, which lie under the peritoneum against the dorsal body wall in the abdominal cavity. Remove the peritoneum from the kidney and observe its shape.

Examine the inner curvature of the kidney and locate the thin-walled duct which leaves the kidney in the center. This is the *ureter*. Follow the ureter to the bladder. The bladder narrows down at its posterior end to form a small duct called the *urethra*. The urethra passes into the pelvic cavity and ends in the urogenital opening ventral to the anus.

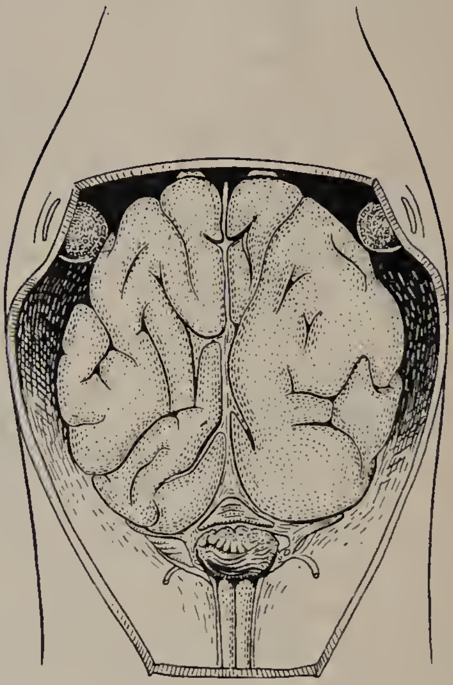


Fig. 38-1-d Brain of fetal pig

In the dissection of the reproductive organs, the technique differs according to the sex of your specimen. Your teacher may outline the two procedures.

Section 5 *Dissection of the brain.* This dissection is difficult and tedious work. Turn the animal so that the dorsal side is up. To expose the brain, remove the skin from the entire skull. The simplest method of exposing the brain with the instruments you have is to cut through the skull near the center, using extreme care not to break the *brain membranes*. After the skull is opened, use the handle of the scalpel to chip away pieces. Do *not* use the blade of the scalpel for chipping. When chipping, hold the scalpel by the side of the handle, *not by the blade*. If bone cutters or heavy forceps or pliers are available, use them in preference to the scalpel handle for chipping. When the brain is completely exposed, you should find two large *cerebral hemispheres* and the *cerebellum* posterior to the *cerebrum*. Unless the brain is removed, you will not find the *medulla oblongata*, although the *spinal cord* can be found.

In Fig. 38-1-d label: **cerebrum, medulla oblongata, cerebellum, spinal cord.**

Interpretation. Discuss the various ways in which the animal you have been dissecting differs from a pig that has been born. Include in your discussion the names and functions of special fetal structures and possible differences in size of organs. ....

....

....

Study of Bone

40-1

Purpose To study the structure and composition of bone and to study the distribution of radioactive phosphorus in a rat.

Bone is well adapted to its functions of support, protection, and providing of a place for muscle attachment. About 65 percent of it is inorganic material in the form of mineral compounds such as calcium carbonate and calcium phosphate. These give bone strength and weight. Cells and gelatinous substances make up the remaining organic matter, which make bone living and somewhat pliable.

A Part 1 EXAMINATION OF EXTERNAL AND INTERNAL STRUCTURE OF A LONG BONE. Although many people think of bone as being dry and nonliving, it is living tissue with an extensive blood supply and in which cells are actively depositing minerals and producing red corpuscles.

Materials. Clean uncooked long bone; longitudinal section of long bone; prepared slide of a cross section of bone; microscope.

Procedure and observations. Examine the long bone carefully. Note the bluish-white covering on the ends. This is the *articular cartilage.* (a) What is its function? ....

....

The enlarged ends of the bones are called the *epiphyses* (sing. *epiphysis*), and the center, or shaft, is called the *diaphysis.* The bone is covered by a thick, double-layered membrane, the *periosteum,* which contains blood vessels, nerves, and bone-forming cells. (b) Explain why bone requires a blood supply.

....

....

Locate areas on the periosteum where muscles might have been attached. (c) Describe these points of attachment. ....

....

....

Now examine the longitudinal section of the long bone with a hand lens. Observe that two types of tissue compose a long bone, *spongy* and *compact.* (d) Which area of the bone is composed almost entirely of compact bone? .... (e) Explain why. ....

.... (f) Which area is composed almost

Longitudinal section of a long bone

entirely of spongy bone? .... (g) Explain why. ....

.... The diaphysis of a long bone encloses the long, cylindrical *medullary canal.* (h) What substance is found in this canal?

.... (i) What is its function?

.... See if you can locate the *endosteum,* which borders the medullary canal. Draw the longitudinal section of a long bone in the space provided and label: **periosteum, articular cartilage, diaphysis, epiphyses, medullary canal,** and **endosteum.**

Compact bone is composed of structural units called *Haversian systems.* Examine a prepared slide of a cross section of ground bone and compare a Haversian system with Fig. 40-1-a. Note the concentric rings. These are called *lamella* and are laid down when bone is formed. Bone cells, called *osteocytes,* are found in the cavities called *lacunae,* which lie between the lamellae. The fingerlike projections from the lacunae are the *canaliculi.* They contain fluids that transport materials from the bone-forming cells and the capillaries. Because of these canaliculi, bone tissue maintains the quality of living tissue in spite of the fact that about 65 percent of it is mineral salts. In Fig. 40-1-a label: **lamella, osteocytes, lacunae, canaliculi.**

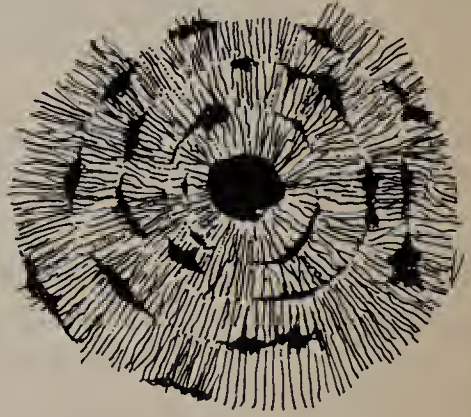


Fig. 40-1-a A Haversian system

B Part 2 DEMINERALIZATION OF BONE. In this Part you will use hydrochloric acid to dissolve the minerals in a bone, leaving the organic materials, which are responsible for the fact that bone is alive.

Materials. Rib bones; scalpel or razor blade; 150-ml beaker; covered dish; tripod; funnel (3-in. diameter); evaporating dish (3 in.); Bunsen burner; filter paper; distilled water; dilute hydrochloric acid; tongs.

Procedure and observations. Put a strip of rib bone in a covered dish. Cover the bone with dilute hydrochloric acid and let it soak for six to eight days. Add fresh acid every two days. After the bone has demineralized (you can bend it at this time), wash it, section it lengthwise, and remove the marrow. With your scalpel or razor blade cut the bone into small sections. Wash the bone again to remove the acid completely. Put the pieces in a beaker with a small amount of distilled water and boil for an hour. Filter the material. Put the filtrate (the liquid that has passed through the filter) in an evaporating dish and heat until dry. Allow it to cool, then examine. (a) Is there any color present? .... (b) If so, what is responsible for the color? ....

(c) What is the characteristic of the filtrate? ....

(d) Why does it have this characteristic? ....

Interpretation. On the basis of your examination, explain how organic matter adapts bone to its function as a supporting organ. ....

....

....

B Part 3 ANALYSIS OF BONE ASH. In this Part you will burn bone to remove the organic matter and study the ash to determine what minerals are present.

Materials. Rib bones; 150-ml beaker; 2 test tubes; evaporating dish; ring stand with ring; Bunsen burner; test-tube holder; filter paper; Hydrion paper; dilute nitric acid; 1% ammonium hydroxide; 1% silver nitrate solution; 1% silver chloride; 1% ammonium carbonate; 1% ammonium chloride; acetic acid; 1% potassium thiocyanate; distilled water.

Procedure and observations. Burn a strip of rib bone in an evaporating dish until an ash is formed. Observe the bone as it is burned. (a) What color appears in the bone and on the sides of the evaporating dish? .... (b) Account for this color.

....

(c) What color is the ash? .... (d) Account for the color.

....

Measure out 1 gm of the bone ash and put it in a beaker. Add a little dilute nitric acid. (e) Describe what happens. ....

.... Add distilled water, stir the mixture until the ash is completely dissolved, then filter. Discard the residue (the part that is left in the filter paper). Add ammonium hydroxide to the acid filtrate (the part that filtered through the paper) until the solution is basic. Use Hydrion paper to test the solution. A solution is basic when the pH is above 7. A heavy, white precipitate of phosphate will be produced by the reaction of ammonium hydroxide with the filtrate. Filter off the phosphates and save the residue. Separate the filtrate into two test tubes. Add a 1% silver nitrate solution to one tube. Silver chloride will precipitate out if chlorides are present. (f) Is a precipitate formed? .... Heat the remainder of the filtrate in the other tube and bring to boiling; then add ammonium carbonate and ammonium chloride *slowly* and simultaneously to the heated solution as long as a precipitate forms. Filter this solution; the residue you obtain is calcium carbonate.

Add acetic acid to the residue on the paper you saved. (g) Does all of the residue dissolve? .... (h) If not, explain. ....

....

If you had residue left, add dilute hydrochloric acid to this material. Add a 1% potassium thiocyanate solution to the resulting solution. (i) Observe and record your observations.

....

A red color indicates the presence of iron.

Interpretation. On the basis of your analysis, describe the mineral content of bone. ....

....

....

C Part 4 RELATIVE DISTRIBUTION OF RADIOACTIVE PHOSPHORUS IN THE ORGANS OF THE WHITE RAT

Problem. How much more phosphorus is normally found in bone than in other tissues?

Phosphorus is found in all the cells of the body. It is an especially important component of bones and teeth, where it is deposited as calcium phosphate. Like the other elements in the body, it is continually being replaced, so that it is essential in the diet.

It is possible to determine the relative amount of phosphorus absorbed in various tissues by injecting a carrier solution containing a radioactive isotope of the element, P^{32}, into a white rat. Although this shows only the amount absorbed and not the total content, the two are usually directly proportional.

Suggested materials. Four white rats; 10 microcuries of P^{32} contained in sodium hydrogen phosphate dissolved in physiological saline as a carrier solution; Geiger counter; planchet; hypodermic syringe (tuberculin type); dissecting set; sensitive balance; enameled pan; small dishes; cage; food for animals; cotton; ether.

Suggested procedure. Weigh each of the rats and record their weights. Use the hypodermic syringe to inject each animal intraperitoneally (into the abdominal cavity) with 2.5 microcuries of P^{32}. After two hours, etherize and sacrifice one of the animals. Dissect it and separate the blood, thyroid and trachea, muscle, bone, lung, spleen, liver, heart, brain, kidney, reproductive organs, and skin. Weigh out 0.2 to 0.8 gm wet fractional parts of each of the organs. Put each of the samples in individual planchets and use the Geiger counter to count each while wet. Use the counts you obtained on the fractional parts of each organ to determine the approximate activity of each organ. Assuming that bone tissue composes 10 percent of the total body weight, calculate the percent of the total amount of P^{32} absorbed by the bones. Calculate the activity in one gram of each tissue or organ and plot on a graph.

Sacrifice a second animal six hours after the injection. Remove the tissues and organs and calculate the activity. Plot your results on the graph you prepared for the first animal.

Sacrifice the third rat at the end of 24 hours and the fourth rat four days after the injection. Separate the tissues and organs and calculate the activity as you did previously. Plot your results on the graph.

Analysis and conclusions. Summarize all data in a table and prepare a graph for the percentage of P^{32} dose absorbed by the bone against time elapsed between injection and dissection. *Be sure to correct your data to allow for radioactive decay of the phosphorus.* Your investigation should help you to understand that the rate of absorption of phosphorus varies with the organ and with time. It should also reveal the extent to which tissues are selective in their absorption of phosphorus.

Purpose To study the contraction of skeletal, smooth, and cardiac muscle under various environmental conditions.

Muscle cells are specialists in motion because they have the ability to contract. In the body, when muscle tissue is supplied with energy from ATP and activated by a nerve impulse, it contracts. In this Investigation you will remove muscles from a freshly pithed frog and use artificial means to study their contraction.

B Part 1 STUDY OF MUSCLE CONTRACTION. Skeletal muscles are those that move parts of the skeleton or support body parts, such as the biceps and triceps and the abdominal muscles. They may be controlled at will. Smooth muscle, on the other hand, forms the walls of internal organs such as the stomach and the arteries and is involuntary. Cardiac muscle is the special tissue found in the heart. It is also involuntary. In this Part you will study the contraction of these three types of muscles taken from a frog.

Materials. Live frogs; jar for frog; cotton; straight probe or pithing needle; forceps; scissors; Petri dishes; dry cell (1.5 volts); electrodes; femur clamp; induction coil; 2 feet five-gauge wire; 2 feet medium-gauge insulated wire; 2 nuts; 5-10 gm weights; ether; Ringer's solution; acetylcholine (1:10,000); adrenalin (1:10,000).

Procedure and observations. Section 1 *Pithing the frog.* Pithing is the process of destroying the spinal cord or entire central nervous system in order to use the animal for experimental purposes. Before pithing the frog, it is advisable to etherize it. Put a piece of cotton saturated with ether in a jar. Put the frog in the jar and leave it there until it becomes limp.

Hold the frog in your left hand so that the head is held firmly between the index and middle finger and the thumb rests on the back. Hold the legs against the palm with your last two fingers. Bend the head down with your index finger until it forms a right angle with the back. Put a dissecting needle on the head at a point on the line that bisects the head. Hold the needle as you would a pencil, and while applying pressure, draw the needle down the head until it reaches the groove at the junction of the cranial cavity and spinal column (see Fig. 40-2-a). This groove is located about midway on a line that runs just posterior to the tympanum. Insert the needle, directing it forward and downward. This will separate the brain and spinal cord. Keep the needle parallel to the head. Insert the needle in the brain cavity and move it from side to side to destroy the brain. Gently touch the cornea of the eye to determine whether the brain has been destroyed. If you have been successful in destroying the brain, the eye reflex will be missing from both eyes.

To complete the pithing, withdraw the needle from the brain cavity, leaving about an eighth of an inch inserted. Turn the needle so that it is directed toward the hind legs and parallel to the back. Locate the soft spot, which is the spinal cord, and direct your needle toward the hind legs. The hind legs will extend and become limp as a result of the stimulation to the spinal cord. When this happens, withdraw the needle.

Section 2 *Comparison of smooth, skeletal, and cardiac muscle activity.* For your study of skeletal muscle use the gastrocnemius, a large muscle in the lower leg of the frog. To remove the gastrocnemius, make a circular cut through the skin anterior to the knee. Using forceps or your fingers, peel back the skin and expose the lower leg. Locate the gastrocnemius, which is the largest muscle on the dorsal side of the shank. To loosen the muscle from those lying beneath, insert a probe under the belly of the muscle and move it back and forth between the knee and ankle. Using your scissors cut the muscle near the tendon of Achilles, as shown

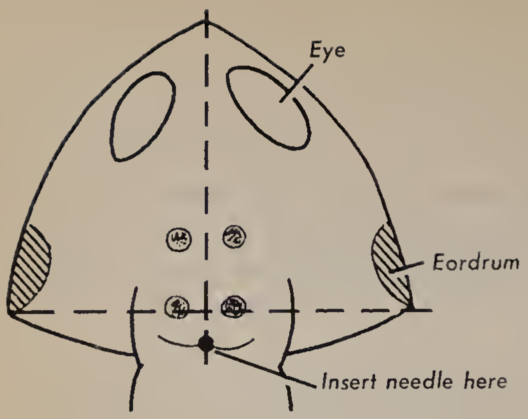


Fig. 40-2-a Inserting the needle for pithing

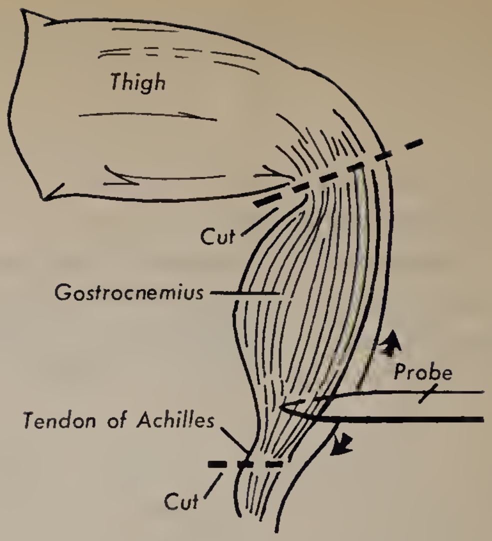


Fig. 40-2-b Excising the gastrocnemius muscle

in Fig. 40-2-b. Lift the muscle by the tendon and cut it from its insertion. Now place the muscle in a Petri dish containing 0.7% Ringer's solution.

Open the abdominal cavity of your specimen and remove the stomach. This is smooth muscle. Place the stomach in the Petri dish with the gastrocnemius muscle.

Open the thoracic cavity to expose the heart. Gently open the pericardial sac. Cut the fine strand of tissue located at the apex of the ventricle. Then with forceps raise the apex and lift the heart up and toward you. Locate the sinus venosus and the two blood vessels leading into it. Cut these two blood vessels. Bathe the heart with the Ringer's solution and use paper toweling to absorb the blood and solution in the cavity. Locate the aorta, a large blood vessel leading from the ventricle. Cut the vessel and remove the heart from the cavity. Rinse the excised heart in Ringer's solution to remove any blood. Then place it in the Petri dish with the stomach and gastrocnemius muscle.

Observe the three muscles carefully. (a) Do all the muscles continue to contract? ....

(b) If not, explain. ....

Now place the gastrocnemius muscle on a paper towel moistened with Ringer's solution. Touch the surface of the muscle with electrodes leading from a battery (dry cell). (c) What happens to the muscle? .... (d) How are skeletal muscles stimulated in your body? ....

Section 3 *Regulation of smooth and cardiac muscle activity.* Put the stomach muscle and cardiac muscle in a separate Petri dish of Ringer's solution. Observe the rate and strength of the contraction of each. (e) Record the rate of contraction for one minute. ....
Add acetylcholine one drop at a time to each of the muscles. At one-minute intervals record on a graph the rate of beat per minute for each level of acetylcholine. Use a different color for each kind of muscle. The number of drops is put on the abscissa, and the rate of contractions on the ordinate. Add the acetylcholine until the smooth muscles go into spasms and the heart ceases to beat. (f) Discuss the responses in both muscles. ....

....

Put the muscle tissues in fresh Ringer's solution and follow the same procedure using adrenalin instead of acetylcholine. Plot a graph for this part. (g) How do your results compare with those you found when you used acetylcholine? ....

....

(h) Discuss the effect these two substances have on the same kind of muscles in the body.

Section 4 *Regulation of skeletal muscle activity.* Cut through the thigh muscle and femur about halfway between the knee and pelvis and leave the portion shown in Fig. 40-2-c. Attach the femur to a femur clamp, as shown in Fig. 40-2-d. Be sure to adjust the tension on the muscle by adjusting the distance between the femur clamp and lever. (i) Explain why tension on skeletal muscles is important?

Remove the insulation from both ends of the wires that make contact with the induction coil and the muscle. Wrap the finest of the wires tightly around the tendon, and insert the other in the muscle, as in Fig. 40-2-d. Put a dish or container under the muscle to catch the Ringer's solution which must be used to bathe the muscle to prevent drying. Regulate the induction coil to deliver a strong enough tetanic stimulus to produce a shock. Increase the stimulus until a maximum response is reached. (j) What does this technique demonstrate?

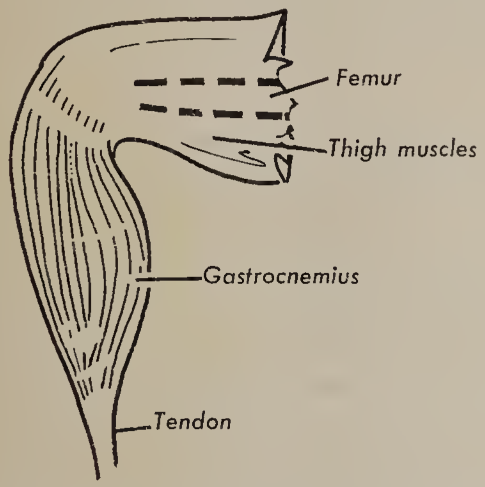


Fig. 40-2-c Gastrocnemius-femur preparation

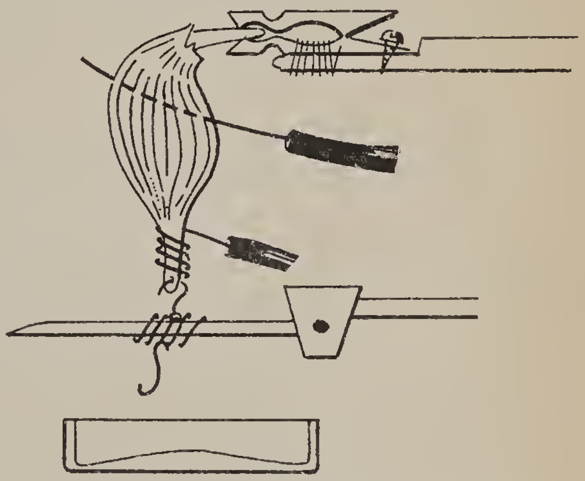


Fig. 40-2-d Setup for demonstrating skeletal muscle cavity

Interpretation. Summarize the activity and regulation of skeletal, smooth, and cardiac muscle that you have observed in this Investigation.

Chemistry of Digestion

41-1

Purpose To determine the action of enzymes on foods and the effect of temperature on this action.

Enzymes are catalytic agents which control the rates of chemical reactions in organisms. There are many kinds of enzymes in your cells, each with a specific chemical function. Digestive enzymes, with which we are concerned in this Investigation, break down complex insoluble food molecules into simple molecules that can be utilized by your body. Digestive enzymes are referred to as extracellular enzymes because they are secreted from the cells in which they are produced and perform their particular chemical activity outside the cell.

A Part 1 ACTION OF DIGESTIVE ENZYMES. In this Part you will conduct controlled experiments to demonstrate the action of digestive enzymes on starch, proteins, and fats. The process will be similar to the digestion of these foods in your digestive system.

Materials. Culture (test) tubes; test-tube rack; test-tube holders; glass-marking pencil or gummed labels; filter paper; litmus paper; funnel; ring stand; crackers, hard-boiled egg white; lard or butter; 2-gm commercial pancreatin; 2-gm commercial pepsin; 0.4% hydrochloric acid solution; sodium carbonate; iodine solution; Fehling's solution; distilled water; dried milk powder; pancreatin powder; olive oil; 5% bile salt solution (sodium taurocholate); water bath.

Procedure and observations. Section 1 *Salivary digestion.* The secretion of the salivary glands contains the enzyme salivary amylase, sometimes called ptyalin. This enzyme acts on starch, converting it to maltose.

With gummed labels (or a glass-marking pencil), number six test tubes consecutively. Put them in a rack. In test tubes 1 and 2 put a small piece of cracker and add enough water to fill the tubes one-third full. Shake the tubes and allow them to stand for a few minutes. Test for the presence of starch with iodine solution in test tube 1, and for the presence of a simple sugar with Fehling's solution in test tube 2. Record your results in the table provided. Indicate the presence of starch or sugar with a plus (+), and their absence with a minus (−). Now rinse your mouth with water and put a small quantity of saliva in test tubes 3 and 4. Test for the presence of starch in 3 and simple sugar in 4. Record your results in the table. Chew a piece of cracker *thoroughly,* but do not swallow it. When the cracker is chewed into a soft mass, put part of it in test tubes 5 and 6. Test for starch in 5 and for simple sugar in test tube 6. Record the results in the table provided.

	Cracker and Water		*Saliva*		*Chewed Cracker*	
	Test tube 1	*Test tube* 2	*Test tube* 3	*Test tube* 4	*Test tube* 5	*Test tube* 6
Starch						
Sugar						

(a) Explain why tubes 1 and 2 contain only water and cracker. ....

....

(b) Explain your results.

Section 2 *Gastric digestion.* Gastric fluid, secreted by the gastric glands, contains hydrochloric acid and the enzyme pepsin. Make a solution of artificial gastric fluid by mixing two grams of commercial pepsin in 0.4% hydrochloric acid solution to make 100 ml of the fluid. Filter this to obtain a clear fluid. Test the resulting solution with litmus paper. Number six test tubes consecutively and put them in a rack. Put a few pieces of cracker in test tube 1, a small piece of hard-boiled egg white in test tube 2, and a small quantity of lard or butter in tube 3. Fill each tube one-third full with the artificial gastric fluid that you have just made. In test tubes 4, 5, and 6, put a piece of each of the same foods, but fill each tube one-third full of plain water.

Allow the six test tubes to stand overnight in a warm place, as near body temperature as possible. Then check the degree of digestion by noting the cloudiness of each solution. Fill in the table provided in Section 3 to show the results by marking a plus (+) to indicate that digestion has occurred, and a minus (−) to show lack of digestion. (c) Explain the results.

(d) Explain why pepsin was added to the hydrochloric acid.

Section 3 *Pancreatic digestion.* Pancreatic fluid, secreted by the pancreas into the small intestine, contains several enzymes essential to digestion. Prepare a solution of artificial pancreatic fluid by mixing two grams of commercial pancreatin and distilled water to make 100 ml of the fluid. Add a pinch of sodium carbonate. Stir and filter to get a clear liquid. Test the solution with litmus paper. Number three test tubes and put them in a rack. Put a few pieces of cracker in test tube 1, a small piece of hard-boiled egg white in test tube 2, and a small amount of lard or butter in test tube 3. Fill each tube one-third full with artificial pancreatic fluid. Save 10 ml of the fluid for use in Part 2.

Allow these test tubes to stand in a warm place overnight. Then check the degree of digestion by noting the extent to which each of the foods has been dissolved. Fill in the table provided to show the results of digestion.

Food tested	*Water*	*Gastric fluid*	*Pancreatic fluid*
1. Cracker			
2. Egg white			
3. Lard or butter			

(e) Explain the results.

(f) Which do you think is more important to digestion, salivary or pancreatic amylase?

(g) Explain your answer.

Section 4 *Litmus milk test.* This test is used to demonstrate that pancreatin contains digestive enzymes that split fats into fatty acids and proteins into amino acids. To prepare the litmus milk, combine 50 gm of dried milk powder and 1 gm litmus powder. Then dissolve 5 gm of the mixture in 45 ml of water.

Put 10 ml of the litmus-milk-powder solution in each of two test tubes. Then add a pinch of pancreatin powder to the solution in one tube. Add water to the second tube to serve as a control. Put the tubes in a warm water bath (40°C) and observe the reaction in the tubes.

(h) Record and explain the results. ....

....

....

Section 5 *Digestion of fat.* In this Section you will demonstrate the relationship of pancreatic lipase and bile. Identify three test tubes as *1, 2,* and *3.* Put 10 ml of water in each of the test tubes, then add a few drops of olive oil. To tubes 1 and 2 add 2 ml of 5% bile salt solution (sodium taurocholate). Add 5 ml of pancreatin solution to tubes 1 and 3. Check each of the solutions with litmus or Hydrion paper. The solutions should be neutral. Place the three tubes in a water bath (40°C) for 30 minutes. Then check the solutions for the formation of acid with the litmus or Hydrion paper. (i) What changes occurred in tube 1? ....

....

(j) in tube 2? ....

(k) in tube 3? ....

(l) Account for the difference. ....

....

A Part 2 EFFECT OF TEMPERATURE ON ENZYME ACTION. Enzymes are more active at body temperature than at extremes of hot and cold. In this Part you will demonstrate the effect of temperature on enzyme action.

Materials. Culture (test) tubes; test-tube rack; refrigerator; incubator; starch suspension; artificial pancreatic fluid (as prepared in Part 1); water bath; Benedict's solution.

Procedure and observations. Prepare a starch suspension and add 10 ml to each of four test tubes. Now add 1 ml of the pancreatic fluid to each of the test tubes. Put one tube in the refrigerator, one in the incubator, and leave one at room temperature. Put the fourth in a water bath at 60°C. Leave the tubes in their respective environments for 30 minutes. Then put 2 ml of Benedict's solution in each tube and heat for 30 seconds. (a) Record your results. ....

....

(b) What effect does temperature have on pancreatic amylase? ....

....

Purpose To study circulation in the web membrane of the frog's foot; to examine blood smears; to study the effect of oxygen and carbon dioxide on blood; to make a comparative study of red blood corpuscles of vertebrates.

All the cells of your body depend on the blood for their existence. The heart, arteries, veins, and capillaries form a transport system in which essential materials are carried to the cells and waste materials are carried away.

A Part 1 BLOOD VESSELS AND CIRCULATION. In this Part you will observe circulation in the webbed membrane between the toes of a frog. If the tail fin of a goldfish or tadpole is used, your teacher will give you slightly different instructions.

Materials. Live frog; microscope; cork board; heavy thread or string; pins; dropping pipette; cheesecloth; paper toweling; absorbent cotton; crushed ice; 0.02% adrenalin solution.

Procedure and observations. Saturate cheesecloth, paper toweling, or absorbent cotton with water. Wrap a live frog in the saturated material and lay it on a cork board. Fasten the frog to the cork board with heavy thread or string secured by pins. Punch a hole in the cork board and spread the web of one foot over the hole. To immobilize the toes, wind thread around each toe and fasten it to the cork board with pins as shown in Fig. 42-1-a. Use a dropping pipette to keep the frog's skin moist during the Investigation. (a) Why is this important? ....

....

Put the board on the stage of your microscope and, using low power, focus on the webbed membrane. Observe the blood vessels in the web. (b) Describe the variation in size of the blood vessels and the rate and direction of blood flow. ....

....

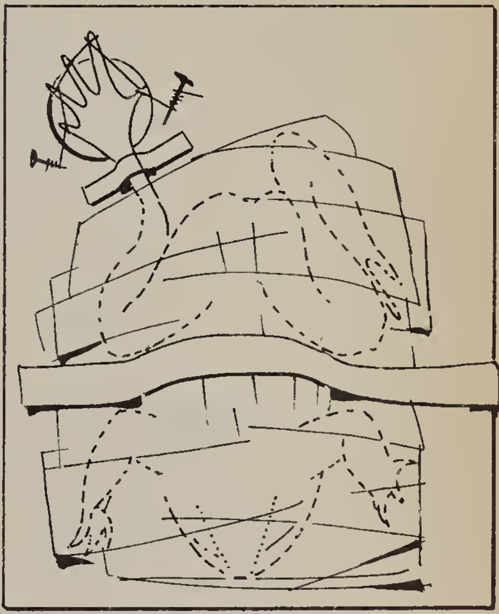


Fig. 42-1-a Setup for the study of circulation in the frog's foot

Survey the membrane under high power. Try to locate the white corpuscles as well as the red ones as they are carried through the capillaries. The diameter of the capillaries can be estimated by using the red corpuscles as a comparison for measurement. The red corpuscles of the frog are 22 microns long, 15 microns wide, and 4 microns in thickness. Or you may use a micrometer in the eyepiece of your microscope, or a plastic ruler laid under the web. (c) What is the estimated diameter of the capillaries? .... (d) What is the function of the capillaries? ....

(e) How do capillaries differ in structure from other blood vessels? ....

....

You may use the frog membrane to investigate the effect of various stimuli on blood circulation. Put some crushed ice on the web and observe the blood vessels. (f) Describe the reaction. .... Remove the crushed ice and allow the membrane to warm to room temperature. Now add warm water (40°C) to the web and observe the blood vessels again. (g) Describe the reaction. ....

.... (h) Observe and record the reaction of blood vessels and circulation to the presence of a drop of a 0.02% adrenalin solution on the web.

....

A Part 2 EXAMINATION OF HUMAN BLOOD. The microscopic examination of human blood reveals that it contains several types of cells. These are more easily studied by staining the cells. In this Part you can use prepared slides of blood smears or you can prepare your own.

Materials. Microscope; prepared slides of blood smears or slides; sterile lancet or needle; Wright's blood stain; alcohol.

Procedure and observations. You can use prepared blood smears or you can prepare your own smears as follows: cleanse the tip of your middle finger with alcohol. Stroke the middle finger toward the tip, pressing as you stroke, until blood is forced toward the tip. Using a sterile lancet or needle, prick the skin near the tip to obtain a drop of blood. (CAUTION: *Do not use the same lancet or needle for more than one student.*) Put the drop on a slide and, using the edge of another slide, spread the blood into a thin smear. Allow the smear to dry for a few minutes. Wipe your finger with alcohol-moistened cotton to prevent infection. When the smear is dry, pass it through the tip of a Bunsen burner flame three times. This will just warm the slide and fix the blood cells in place. If Wright's stain is available, add a few drops of it to the smear. After two or three minutes, rinse the stain from the slide in water, dry, and examine under the high power of your microscope. If you do not have Wright's stain, examine the smear immediately. The cells near the edge of the smear are more easily seen because they are widely separated. Compare the corpuscles you see with those shown in your textbook. In the space provided, draw a group of red corpuscles and several white corpuscles, showing variation, if possible, in the latter. Label: **each type of corpuscle.**

Red and white blood corpuscles

B Part 3 EFFECT OF O_2 AND CO_2 ON BLOOD. The veins of your arm and hand appear blue, but when blood is removed from the veins, it is not blue. What is the color of blood in the blood vessels? Does the blood change color under certain conditions?

Materials. Fresh whole citrated blood; two 250-ml Erlenmeyer flasks; 2 test tubes; ring stand and clamp; 2 one-hole stoppers to fit flasks; glass tubing; Bunsen burner; bicycle pump; manganese dioxide; potassium chlorate; sodium bicarbonate; dilute hydrochloric acid.

Procedure and observations. Fill two 250-ml Erlenmeyer flasks or other glass containers half full of the fresh blood. Insert glass tubes bent at right angles into one-hole stoppers that fit the flasks. Be sure the openings of the tubes are well below the level of the blood. When the flasks are stoppered, connect a bicycle pump to the tube of one flask and slowly and carefully pump air into the flask for five minutes. Breathing normally, blow air from your lungs into the second flask for one minute. Compare the color of the blood in the two flasks. (a) What color is the blood into which the air was pumped?

.... (b) into which you breathed? ....

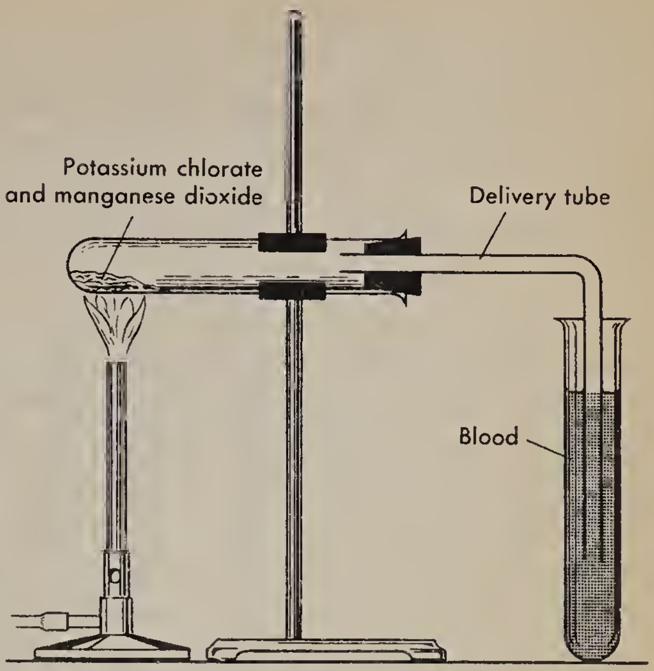


Fig. 42-1-b Oxygen generator

Since the air from the bicycle pump and your lungs is a mixture of gases, you should do a second experiment to determine which component of the air was responsible for the color change. Put dry manganese dioxide and potassium chlorate in a test tube. Then fill a second test tube two-thirds full of fresh blood and set up the apparatus as shown in Fig. 42-1-b. Heat the mixture and allow the generated oxygen to bubble through the blood for three to five minutes. Note carefully any color change. (c) What happens? ....

.... (d) Explain. ....

....

Compare the color of the blood in the test tube with that still in the jar. (e) Account for any difference in color. ....

....

Put some bicarbonate of soda and dilute hydrochloric acid in a test tube and bubble the generated carbon dioxide through the blood. (f) What effect does the carbon dioxide have on the blood? .... Put a drop of oxygenated and deoxygenated blood on a piece of filter paper. (g) Compare their color. ....

....

Interpretation. On the basis of your observations, explain why blood flowing from a vein other than the pulmonary vein would be a darker red than blood flowing from an artery other than the pulmonary artery. ....

....

....

....

Venous blood appears blue when seen through the skin. Can you explain this in terms of absorption of light? ....

....

C Part 4 COMPARATIVE STUDY OF THE RED BLOOD CORPUSCLES OF VARIOUS VERTEBRATE ANIMALS

Problem. How do the red corpuscles of various vertebrates differ from one another?

Since the red blood corpuscles function as carriers of oxygen, an animal's metabolic activities relate to the amount of oxygen its red blood corpuscles can carry. The amount of oxygen the corpuscles can carry is in turn related to the presence or absence of nuclei.

Suggested materials. Blood smears of fish, frog, reptile, bird, and mammal; microscope; slides; hypodermic needle.

Suggested procedure. Various methods may be used to obtain sufficient blood from smears from the animals suggested. Blood may be taken from a gill or from the heart of a freshly sacrificed fish by drawing it into a hypodermic needle. For frog and reptile (lizard or snake) blood, it will probably be necessary to sacrifice the animal and remove blood from the heart or a large blood vessel with the hypodermic needle. For the bird, draw a small amount of blood from a vein of the wing, using a pigeon or a chicken. Similarly, blood may be obtained from a vessel in the leg of a mammal, such as a rabbit, guinea pig, or hamster. If a living animal is used, be sure to cleanse the skin with alcohol and use a sterile hypodermic needle in obtaining blood. You may use your own blood as a sample of mammalian blood. Prepare blood smears as directed in Part 2 of this Investigation. Examine the smears under high power. Note the differences in red blood corpuscles of the various organisms. Determine the presence or absence of a nucleus, how large the nucleus is in comparison to the size of the cell, and variations in intensity of red coloration.

You may also wish to determine whether there are differences in white corpuscles of the animals. You can study these by following the directions for using Wright's blood stain as directed in Part 2.

Analysis and conclusions. Record your observations as a series of drawings. Your examination of the red corpuscles should help you to explain why certain of the animals are cold-blooded and others are warm-blooded.

Purpose To learn the technique for typing blood; to study blood clotting time; to determine the effect of environment on clotting; to study the nature of a blood clot.

Proteins, although they make up only seven percent of the plasma, are important components of blood. One of them, fibrinogen, is essential for clotting. Other proteins are antibodies which counteract foreign proteins, thus protecting the body against disease.

B Part 1 BLOOD TYPING. The agglutination reaction is used to determine blood types. Agglutination occurs when blood from certain individuals is mixed with the blood of individuals with a different blood type. The reaction resembles the agglutination that occurs between antibodies and bacteria. In blood, however, agglutination occurs between a protein in the red corpuscles of the donor and a protein in the serum of the recipient. The protein in the red corpuscles is called an *agglutinogen*, either *A* or *B*, and the protein in the serum is called an *agglutinin*, either *anti-a* or *anti-b*. Blood types designated as *A*, *B*, *AB*, or *O* are determined by the presence of either or both of the agglutinogens, and, in the case of type *O*, by the absence of both. Your blood serum contains an agglutinin which will cause agglutination of corpuscles that are foreign to it. This relationship is shown in the table below. *Note that no blood contains serum proteins capable of agglutinating its own red corpuscles.*

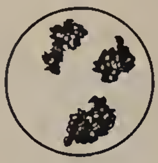
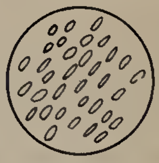
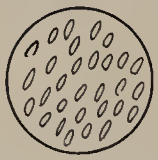
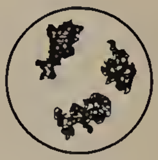
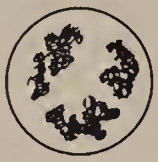
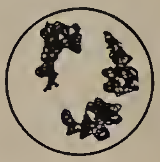
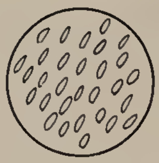
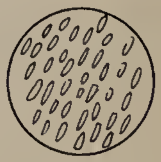
Blood Type	*Corpuscle Protein (Agglutinogen) Present*	*Serum Protein (Agglutinin) Present*
A	*A* corpuscle protein	Anti-b serum protein
B	*B* corpuscle protein	Anti-a serum protein
AB	*AB* corpuscle protein	Neither serum protein
O	Neither corpuscle protein	Anti-a and anti-b serum protein

Materials. Microscope; blood lancet; Rh typing box; slide; hemoglobin scale; glass-marking pencil; white filter paper; serum pipette; toothpicks; cotton; alcohol; anti-a serum; anti-b serum; anti-RhD serum.

Procedure and observations. The test for blood types is made by mixing suspensions of the red corpuscles with various serums, each of which contains a particular agglutinin. If any serum causes agglutination, the red corpuscles contain an agglutinogen which the serum protein agglutinates. Note that agglutination occurs when the serum and red corpuscle proteins are of the same letter.

The technique for blood typing is easy to do. Divide a clean microscope slide with a glass-marking pencil. Identify one side of the slide as *A* and the other side as *B*. Cleanse the tip of the middle finger with alcohol. Stroke the middle finger so as to force blood toward the tip. Then quickly prick the finger with a sterile lancet. Put a drop of blood on both sides of the slide. Put another drop on a piece of white filter paper and compare it with a hemoglobin scale. (a) What is the hemoglobin content of your blood? .... Wipe your finger with alcohol-moistened cotton to prevent infection.

Put a drop of the anti-a serum on the side of the slide identified as *A*. Avoid touching the drop of blood with the serum pipette. Add a drop of anti-b serum to the side of the slide identified as *B*. Using separate fresh toothpicks, stir the anti-a and anti-b mixtures. Be sure to allow sufficient time for agglutination to occur. Then examine the mixtures for agglutination, and compare the results with the compatibility chart below. If the B side shows agglutination, the blood type is *B*. If agglutination occurs only in A, the blood type is *A*. If agglutination occurs in both A and B, then the blood type is *AB*, and lack of agglutination in either indicates type *O*.

Blood Type	*Serum*	
	Anti-a	*Anti-b*
A		
B		
AB		
O		

(b) What is your blood type? .... Tabulate the blood types of all the students in your class in the table provided.

Blood type	*Frequency in U.S. population*	*% Frequency in class*
A	40%	
B	10%	
AB	4%	
O	46%	
Rh+	85%	
Rh–	15%	

Another antigen present in the blood of certain individuals is known as the *Rh factor*. This factor is present in 85 percent of the population. Those who have the factor are called *Rh positive*, and those persons who lack the factor are termed *Rh negative*.

Using the same technique for obtaining blood, test for the Rh factor. Put a drop of blood on a clean microscope slide. Then add a drop of anti-RhD serum to the drop. Stir the mixture with a clean toothpick. If agglutination occurs, identify the individual as Rh positive. If there is no agglutination, identify the individual as Rh negative. Record the results in the table provided. (c) Does the percentage of distribution of blood types approximate the national average? .... (d) Explain. ....

....

Blood typing and compatibility testing is very important when an individual requires a transfusion. The important factor to be considered is whether the donor's red corpuscles will be agglutinated by the recipient's serum. In the table below, show compatibility with evenly diffused red corpuscles and agglutination with irregular masses of corpuscles.

Red Corpuscle Factor (Agglutinogen)	*Serum Factor (Agglutinin)*			
	A (Anti-b)	*B (Anti-a)*	*AB (None)*	*O (Anti-a and anti-b)*
(A) Anti-b				
(B) Anti-a				
(AB) None				
(O) Anti-a and anti-b				

Interpretation. Explain why type-AB blood is sometimes called the "universal recipient" and type O is sometimes called the "universal donor." ....

....

....

....

Explain why Rh-negative blood can be safely transfused into an Rh-positive individual. ....

....

B Part 2 STUDY OF BLOOD CLOTTING. The complex process of blood clotting is essential to health and life. Clotting begins when distintegration of the platelets releases thromboplastin. This reacts with prothrombin and calcium to form thrombin. Thrombin changes fibrinogen to fibrin, which is a network that forms the clot. If any of these substances is missing, clotting will not occur.

Materials. Living rat, mouse, hamster, or chicken; microscope; 6-inch length of glass tubing; fish-tail burner; scalpel; blood lancet; syringe; test tube; shallow dish; slide; cover glass; cotton; dissecting needle; ice; water bath; alcohol; sodium oxalate; calcium chloride.

Procedure and observations. Section 1 *Blood clotting time.* Prepare some capillary tubes by heating a 6-inch length of glass tubing in a fish-tail burner until soft. Pull the hands apart quickly to draw the tube into a capillary tube of at least four inches in length. Score each capillary tube in several places. Cleanse a finger with alcohol and pierce the skin with a sterile blood lancet. Press the finger so as to obtain a good-sized drop of blood. Slant the capillary tube downward from the drop of blood and allow the tube to fill. After four minutes, break the tube at the first scored mark. If clotting has occurred, the blood will pull out of the tube. If clotting has not occurred, wait two minutes and repeat. This should be done at two-minute intervals until clotting has taken place. Clotting time is the interval between filling of the tube and clotting. (a) What was the clotting time for your blood? ....

Section 2 *The effect of various factors on blood clotting.* Using a syringe, withdraw 2 ml of blood from a vein at the base of the tail or a leg of a mouse or rat or from the lower side of the wing of a chicken. Put the blood in a test tube that has been chilled in a refrigerator and observe what happens. Put a drop of the chilled blood on a microscope slide, cover with a cover glass and examine under high power of the microscope. (b) Record your results.

....

Now put the test tube in a hot water bath and observe the results carefully. Examine a drop of this blood under the microscope. (c) Record your results. ....

....

....

....

Withdraw another 2 ml of blood from the specimen. Add a pinch of sodium oxalate to the blood. (d) Observe what happens and record the data. ....

.... Wait 5 minutes and add a pinch of calcium chloride to the solution and observe what happens. (e) Record your results. ....

Sodium oxalate prevents clotting of blood by reacting with calcium salts normally present and essential in the clotting reaction. The addition of calcium chloride speeds the clotting by supplying additional calcium ions which unite with prothrombin in the clotting process.

Withdraw 2 ml of blood from an animal. Put 1 ml in a small test tube and 1 ml in a shallow dish. (f) Compare clotting time of the blood in the two containers. ....

.... (g) Explain the result.

....

....

Section 3 *Study of a blood clot.* Cleanse a finger with alcohol and pierce the skin with a sterile lancet. Press the finger so as to obtain a good-sized drop of blood. Put the drop on a clean slide and watch for clotting to take place. When the clot has formed, run a needle through the drop and lift out the clot. Put the clot on a microscope slide and examine it under high power. (h) Record your observations. ....

....

(i) What are the needlelike fibers? ....

Interpretation. Summarize the effects of temperature, sodium oxalate, calcium chloride and exposure to air on blood clotting. ....

....

....

....

....

The Heart 42-3

Purpose To examine the structure of the heart and the great vessels extending to and from its chambers and to conduct a microscopic examination of heart muscle.

The heart is the center of the vascular system. Every cell of your body depends on the rhythmic contractions of this remarkable pump. Deprived of a continuous supply of nutritive materials, oxygen, and other essential materials and constant removal of metabolic wastes, your cells could survive but a few minutes.

The human heart, like that of other mammals as well as birds, consists of four chambers. Thus, it is a double pump, the right side receiving blood from the systemic (body) circulation and forcing it out through the arteries of the pulmonary (lung) circulation. The larger and more powerful left side receives blood from the lungs and pumps it through the extensive arterial system of the systemic circulation.

Every second of your life the muscles of this powerful organ contract. Unlike all other muscles, the cardiac muscle composing the heart wall rests only between beats. Yet, even with this constant exertion, the average normal heart can continue its work 70 years or more.

A Part 1 STRUCTURE OF THE HEART. The examination of the heart, its chamber and valves, and the great vessels leading to and from it will give you a much greater understanding of heart action. A large heart, such as a hog, beef, or sheep heart is an excellent subject for examination and dissection.

Materials. Hog, beef, or sheep heart; heart model or chart of the heart; dissecting probe; scissors; scalpel; metric ruler.

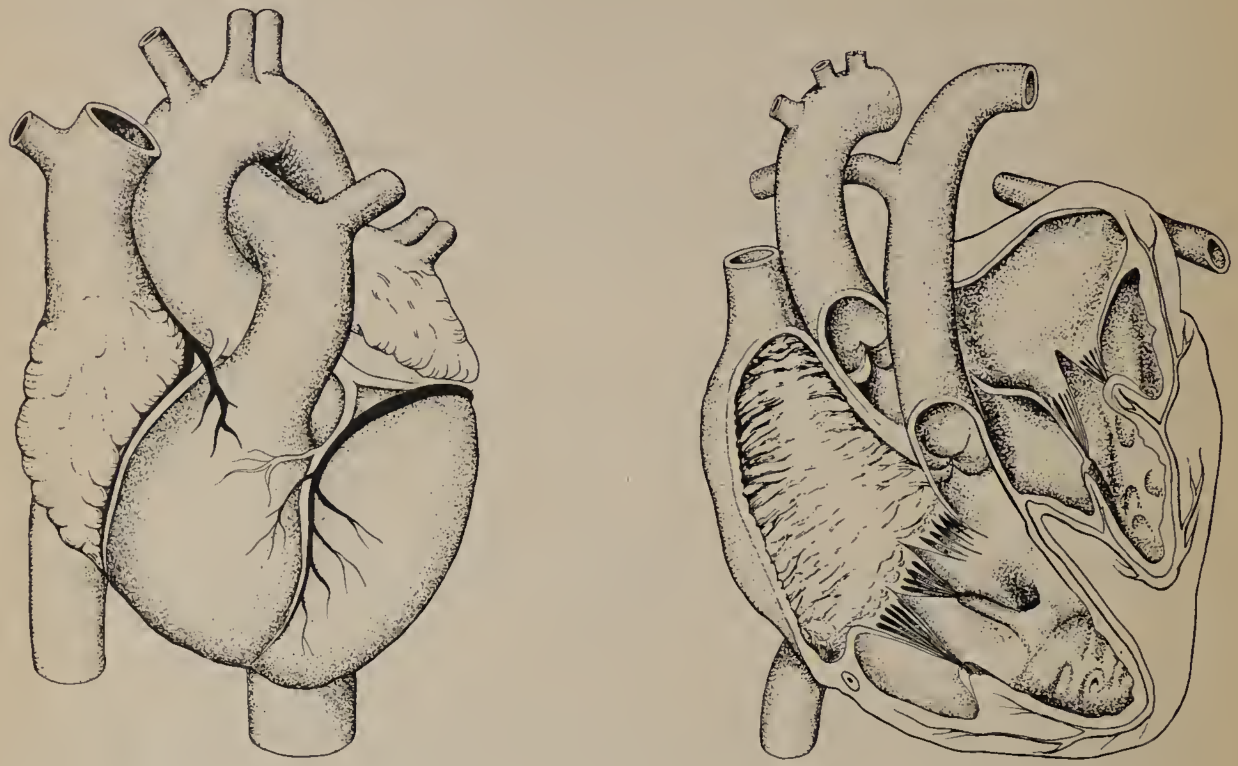
Procedure and observations. Examine the front (ventral) and back (dorsal) surfaces of the heart. (a) Describe its general shape. .... While viewing the front side, locate the region of the right and left *atria* and the right and left *ventricles*. Insert your probe into the *superior* (anterior) and *inferior* (posterior) *venae cavae*. (b) Into which heart chamber do they empty? .... Measure the diameter of each of these great veins: (c) superior vena cava ....; (d) inferior vena cava. .... (e) Describe their walls. ....

....

Examine the back side of the heart and locate the *pulmonary veins*. (f) Into which chamber do they empty? .... Again viewing the front side of the heart, locate the *pulmonary artery*. (g) From which chamber does it arise? .... (h) Measure the diameter of the pulmonary artery. .... Locate the ***aorta***. (i) From which chamber does it arise? .... (j) Measure the diameter of the aorta. .... (k) Compare its walls with those of the venae cavae. ....

Examine the front surface of the ventricles and find the right and left *coronary arteries* and *veins*. (l) What important function is performed by these vessels of the coronary circulation? ....

Compare your heart specimen (or model) with the view of the front of the heart shown in Fig. 42-3-a. Label: **right atrium, left atrium, right ventricle, left ventricle, apex of heart, superior vena cava, inferior vena cava, pulmonary artery, aorta, right coronary artery, right coronary vein, left coronary artery, left coronary vein.**



Ventral surface Longitudinal section

Fig. 42-3-a Human heart

Sectioning a heart to expose the four chambers is difficult. In most hearts, the right chambers lie somewhat anterior to the left chambers. This forward rotation of the right heart prevents sectioning across the heart with a single lateral cut. Furthermore, it is difficult to section the heart longitudinally without damaging the bases of the arteries and veins. For these reasons, it will be necessary to proceed carefully and make several incisions to section the heart longitudinally.

Using your scalpel, cut into the right ventricle at the base of the pulmonary artery. This incision should be oblique and through the approximate mid-line (front to back) of the right ventricle. Continue the incision along the base of the aorta and cut obliquely downward to the tip or apex of the heart. Removal of this portion of the heart wall should expose the right and left ventricles. It may be necessary to make additional cuts to expose these chambers, however.

Locate the approximate center of each atrium and section it laterally, using care not to damage the venae cavae and pulmonary veins.

(m) Compare the sizes of the right and left ventricles. ....

.... (n) Measure the thickness of the wall, or *myocardium,* of the left ventricle. .... (o) Compare the myocardium thickness of the two ventricles. .... Does the thickness of the myocardium vary in different areas of the heart? (p) Explain. ....

.... (q) Locate and describe the septum. ....

.... (r) Describe the appearance of the *endocardium,* which lines the heart chambers. ....

....

The sectioned heart should show part or all of the structure of the heart valves. The *atrioventricular* (a-v) valves guard the openings of the atria into the ventricles. These valves are composed of fibrous flaps which project downward. The tips of the flaps are anchored to the ventricle wall by tendonlike strands attached to projections, called *papillae.* The left atrioventricular, or *bicuspid valve,* is composed of two cusps, or flaps. The corresponding *tricuspid valve* on the right side has three cusps, or flaps.

Other valves, the *semilunar valves,* are situated at the base of the pulmonary artery and aorta. These valves consist of three cup-shaped structures which open with pressure from below (into the arteries) but close tightly and prevent backflow of blood from the arteries into the ventricles. Probe into the base of the pulmonary artery and find the *pulmonary* (right s-l) *valves.* Find the *aortic* (left s-l) *valves* at the base of the aorta. You should be able to open these valves with your probe.

In the diagram of the sectioned heart in Fig. 42-3-a, label: **right ventricle, left ventricle, right atrium, left atrium, bicuspid valve, tricuspid valve, aortic valve, septum, myocardium, endocardium.**

A Part 2 **STUDY OF CARDIAC MUSCLE.** The muscle composing the heart wall is different from any other muscles of the body. The cells are short, quadrangular, and joined end to end. Cells of cardiac muscle branch freely and join one another to form a network. This union of cells is essential in conducting impulses through the heart wall. Cardiac muscle cells have large, centrally located nuclei and faint cross markings, or striations. Prominent disks cross the fibers at regular intervals. These characteristics of cardiac muscle are evident when fibers are seen under a microscope.

Materials. Piece of heart muscle taken from the wall of a ventricle; microscope; scissors; probe; dissecting needles, microscope slide; cover glass; methylene blue.

Procedure and observations. Cut a small piece of heart muscle (¼ in. square) and put it on a microscope slide in a drop of water. Using dissecting needles, spread the fibers apart. Add a drop of methylene blue stain and a cover glass. Examine all parts of the muscle with the low power of your microscope. Find an area in which the fibers are spread sufficiently to show cell details. Examine under high power. Draw several cells in the space provided. Label: **nucleus, striation, branch, disk.**

Cardiac muscle cells (H.P.)

Purpose To chart the path of a spinal reflex action and to demonstrate a spinal and a cerebral reflex action.

Reflex actions are responses to stimuli that occur automatically and extremely rapidly. They are protective in nature. Your reflex actions have prevented injury and may have saved your life on more than one occasion. Generally, a reflex action involves a sensory neuron, a central or connecting neuron in the spinal cord or in the brain, and a motor neuron.

A Part 1 THE PATH OF A SPINAL REFLEX. You have undoubtedly touched a hot stove or stepped on a nail or other sharp object and jerked away before realizing what had happened. The sudden response to the stimulus was a spinal reflex action. Receptor endings of a sensory neuron in the skin received the stimulus. An impulse traveled along the dendrite of the sensory neuron to its cell body in a ganglion outside of the spinal cord, then along the axon and into the spinal cord to nerve endings in the posterior horn. Here the impulse was received by a connecting neuron and was transmitted to a motor neuron, the cell body of which lies in the spinal cord. The impulse was transmitted along the axon of the motor neuron leading from an anterior horn of the spinal cord to a distant muscle, where a response occurred as a muscle contraction. Several nerves and muscles would be involved in jerking a finger from a hot stove.

Materials. Colored pencils.

Procedure and observations. Figure 44-1-a shows the neurons involved in a spinal reflex action. Indicate the ***stimulus*** on the surface of the skin. Then label the **receptor endings** of the sensory neuron, its long **dendrite,** and its **cell body,** located in a spinal ganglion. Label the **posterior horn** of the spinal cord, the **central neuron,** motor nerve **cell body, axon,** and **nerve endings** along a muscle fiber. Indicate the muscle as the place of *response.* Show the path of the impulse along the sensory neuron with a red arrow, along the central neuron with a green arrow, and along the motor neuron with a blue arrow.

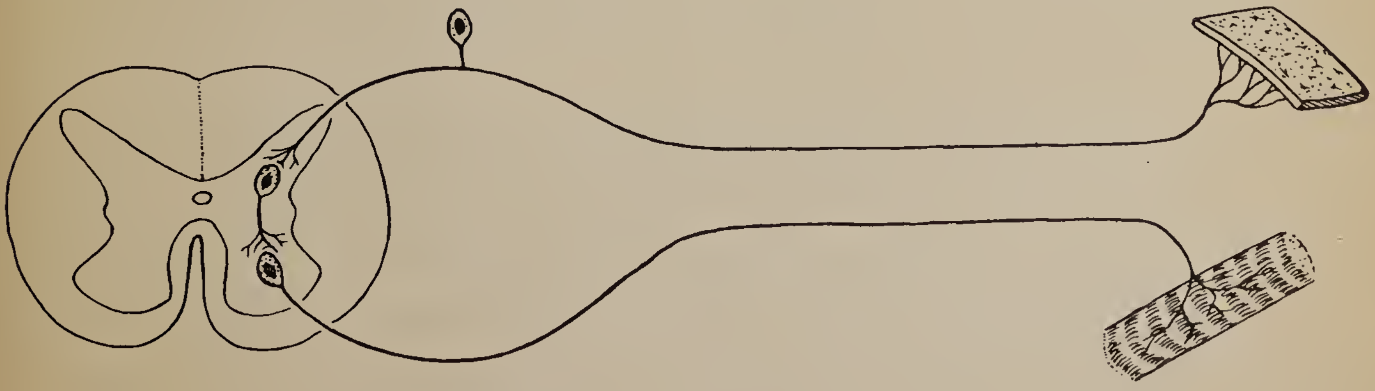


Fig. 44-1-a Path of a spinal reflex action

A Part 2 DEMONSTRATING A SPINAL REFLEX ACTION. The knee jerk is a familiar example of a spinal reflex action and is easily demonstrated.

Materials. No materials or apparatus required.

Procedure and observations. Seat a member of your class on a table so that his lower legs swing free. Using the blunt edge of a ruler or the edge of your hand, strike a point just below the kneecap (the patellar ligament) and note the jerk that follows. (a) Estimate the reaction time between striking the knee and the muscle responses. .... (b) Can the subject prevent the leg from jerking when the knee is struck? ....
Try to fool the subject by starting to strike the knee without actually touching it. (c) Does the leg jerk? .... (d) If the leg jerks, would you consider this a reflex action? .... (e) Explain. ....

....

Repeat the process with the knee bent and straightened. (f) At what angle of flexion of the lower leg does the response seem to be strongest? .... (g) Explain.

....

A Part 3 DEMONSTRATION OF THE PUPIL REFLEX. When you go from an area of one light intensity to another, the iris of your eyes makes an automatic adjustment in the size of the pupils of your eyes. This is another example of reflex action.

Materials. Flashlight.

Procedure and observations. Notice the size of the pupils of the member of the class who will act as a subject for the demonstration. Have him cover his left eye with his hand for three minutes. Notice the pupil immediately as he removes his hand. (a) How does it compare in size with that of the pupil before covering? .... Shine a flashlight immediately into the left eye. (b) Describe the change in the pupil. ....
.... (c) Account for the changes that you observed. ....

....

....

Sensory Receptors 44-2

Purpose To locate and study some of the body receptors.

In addition to our sense organs for sight and hearing, we have sensory receptors specialized for taste, smell, touch, heat, cold, pressure, and pain. The receptors for taste are found only on the tongue and those for smell only in the nose. The other receptors are found all over the body.

A Part 1 THE CHEMICAL SENSES—TASTE AND SMELL. Taste and smell are chemical senses because they result from chemical stimulation of the specialized receptors. Smell receptors are stimulated by gases, while taste receptors are also sensitive to solids and liquids. Since the only four sensations we can actually taste are sour, sweet, bitter, and salty, much of what we call taste is really smell.

Materials. Onion; apple; cotton swabs; paper towels; sugar cubes; sugar solution; salt solution; lemon juice; quinine solution; magnesium sulfate (Epsom salts).

Procedure and observations. Wipe your tongue dry with a clean paper towel. Place a cube of sugar on your tongue. (a) Can you taste it? .... Leave it there for a few minutes. (b) Describe what happens. ....

....

Rinse your mouth with water and wipe your tongue dry a second time. Have your partner touch it with a fresh cotton swab that has been dipped in sugar solution. As the various areas of your tongue are slowly swabbed, note the one in which you detect sweetness most readily. Draw an outline of the tongue in the space provided and indicate this area.

Wipe the tongue dry, discard the swab, and use a fresh swab dipped in salt solution. Chart the area of the tongue that reacts to salty flavors most readily. Repeat the test with lemon juice and then quinine solution. Chart the results on the tongue in the space provided. You now have charted the tongue by locating receptors for sweet, salty, sour, and bitter sensations. (c) Are they located in the same place on your partner's tongue? ....

The taste buds in some areas recognize the substance as being of one taste and other areas recognize it as being another taste. To demonstrate this phenomenon, wipe the tongue dry and have your partner swab the different areas with a cotton swab that has been dipped in a magnesium sulfate solution. (d) Describe your taste sensation. ....

Taste areas of the tongue

....

Certain substances have a distinct odor, but when tasted have an entirely different taste. You can test this by using vanilla extract. (e) Describe the odor of vanilla. ....

.... Now put some on a cotton swab and taste it.

(f) How does it taste? ....

It is often difficult to distinguish certain foods if you cannot smell them. Test this by using samples of onion and apple that are cut into small pieces. Blindfold members of the class. Have them hold their nose and then give them some of the apple. After they have swallowed the apple, give them some onion. (g) Could they determine which one was apple? ....

(h) onion? .... (i) Explain the results. ....

....

A Part 2 **THE SENSATIONS OF THE SKIN.** All areas of the skin are not equally sensitive to all stimuli. In any given skin area there are certain portions, often close together, that can recognize only one kind of sensation.

Another characteristic of the skin receptors is that the degree of your ability to recognize two distinct sensations when your skin is stimulated at two points varies with the area of the skin. In many areas the nerve endings sensitive to touch are so close together that you identify two sensations as one.

Materials. Dissecting needles.

Procedure and observations. Usually when two areas of the skin are stimulated by two different objects, two sensations are felt. However, when these two areas are brought close together, so they can be stimulated by one object, only one sensation is felt.

A student who acts as the subject should cross the middle finger over the index finger as shown in Fig. 44-2-a. Then blindfold the subject. Move the point of a probe or pencil along the angle formed by the fingers. Ask the subject how many objects he felt. (a) Record his answer.

....

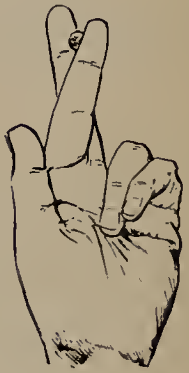


Fig. 44-2-a Finger positions for experiment

Using two dissecting needles, locate the sensitive areas on your partner's arm, hand, finger, and neck. Have your partner look away. Place the points of the needles eight inches apart on the inner surface of the arm. Move the needles closer and farther apart. Ask your partner if he feels one or two needles. (b) How far apart were the needles when he recognized two sensations as one? .... Repeat the process on the neck. (c) What was the distance between the two needles when he recognized only one sensation instead of two? ....

Place the new needles three inches apart in the palm of the hand. Move them closer together or farther apart until your partner recognizes only one sensation. (d) What was the distance between the two needles? .... Repeat the process on the inner surface of the index finger. (e) What was the distance between the two needles when he recognized only one? .... (f) In which area of the skin do the pressure receptors appear to be farthest apart? ....

.... (g) closest? ....

Hearing and Equilibrium 44-3

Purpose To study the structure of the ear, to determine the function of its parts, and to study the nature of equilibrium.

Next to your ability to see, hearing is probably your most important special sense. The human ear converts sound waves into nerve impulses which result in the sense of hearing.

A Part 1 STRUCTURE OF THE EAR. In this Part you will study the ear and determine the role each of its structures plays in the mechanism of hearing.

Materials. Model of the ear.

Procedure and observations. Examine a model of the ear or refer to Fig. 44-3-a. Study all of the parts and label: **outer ear, middle ear, inner ear, eardrum, Eustachian tube, auditory nerve, cochlea, semicircular canals.** (a) What is the function of the external ear? .... (b) How is the middle ear connected with the throat? ....
(c) Why is it necessary to have a connection between the middle ear and throat? .... (d) What structure separates the external and middle ear? ....

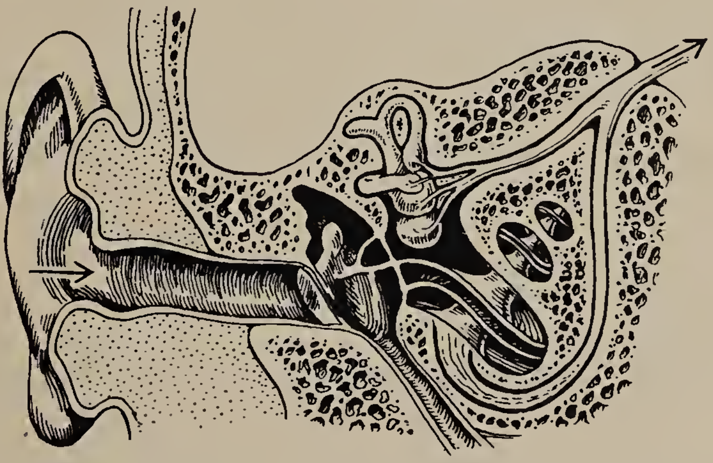


Fig. 44-3-a Section through the ear

(e) Name the three small bones in the middle ear. .... The cochlea and semicircular canals are located in the inner ear. (f) What is the function of the cochlea? .... (g) The semicircular canals? .... (h) What necessary structures line the cochlea? ....

A Part 2 EQUILIBRIUM REACTIONS IN MAN. Since the organs of equilibrium have no known anatomical relation to the cerebral cortex, equilibrium is not classified as one of the senses. Equilibrium is maintained by the semicircular canals together with impressions from the muscles.

Materials. Revolving chair; blindfold.

Procedure and observations. Let one student be the subject in this demonstration. The subject sits in a revolving chair and focuses on a distant object in front of him. Rotate the chair 10 times in 20 seconds to the right. Watch the eyes of the student and note any movements they make. (a) Record what happened. ....

.... (b) Ask the subject what sensations he experienced and record these. ....

.... (c) Compare the sensations experienced by the subject with what you observed. ....

Blindfold a second subject who is seated in the revolving chair. The subject extends his arm directly in front of himself and touches the forefinger of a second student with his forefinger. The subject then raises his arm above his head and lowers it quickly to touch the same point. (d) Does he point to the examiner's forefinger? ....

(e) If not, where is he pointing? ....

(f) How does this demonstration illustrate the importance of vision in orientation of position?

....

....

Now have the subject put his arms on the arms of the chair. Rotate the chair ten times to the right in ten seconds. When the chair stops, have the subject extend his arm so an examiner can touch his forefinger. He then raises his arm and quickly lowers it to touch the forefinger of the examiner. (g) Does he point to the examiner's finger? ....

(h) If not, where does he point? .... (i) Compare the results of this experiment with the previous one. ....

....

You can demonstrate the relationship of the semicircular canals to equilibrium by doing the following experiment. Blindfold a seated subject, have him bend forward and support his head between his knees so as to form a 90° angle. Rotate the chair ten times in ten seconds. Have the subject sit up immediately after the chair is stopped. (j) To what side does he fall?

....

Repeat the previous experiment, but have the subject lean his head toward the right shoulder, returning it to a vertical position on stopping. Test as before by turning the chair ten times. Have him raise his arm above his head and lower it to point at the forefinger of the examiner. (k) In what direction did he point in relation to the subject? ....

Purpose To study the structure of the mammalian eye, to determine the function of each part, and to study the mechanism of vision.

Your eyes are remarkable sensory receptors. They enable you to see in dim light as well as in bright light, and to see black and white and color. You can focus on distant objects and shift your focus to see close objects clearly. In addition to the field of distinct vision, your eyes provide a much larger area of peripheral vision in which objects, while visible, are not distinct.

A Part 1 STRUCTURE OF THE EYE. In this Part you will study the anatomy of the mammalian eye from a chart or model. If cow or sheep eyes are available, your teacher may have you omit this and proceed to the dissection in Part 2.

Materials. Model or chart of the eye showing muscles.

Procedure and observations. There are six sets of muscles that attach the eye to the bony socket. Locate the **superior rectus** muscles on the dorsal surface of the eye. When these muscles contract, the eye turns upward. Locate the **inferior rectus** muscle on the ventral surface of the eye. (a) What is its function? ....

Locate the **lateral rectus** muscle on the outside. (b) What function does it serve? ....

.... Find the **medial rectus** muscle on the inside surface. (c) What is its function? ....

.... Locate the **superior** and **inferior oblique** muscles.

(d) What is the function of the oblique muscles? ....

.... Label the muscles on the diagrams of the eye in Fig. 44-4-a.

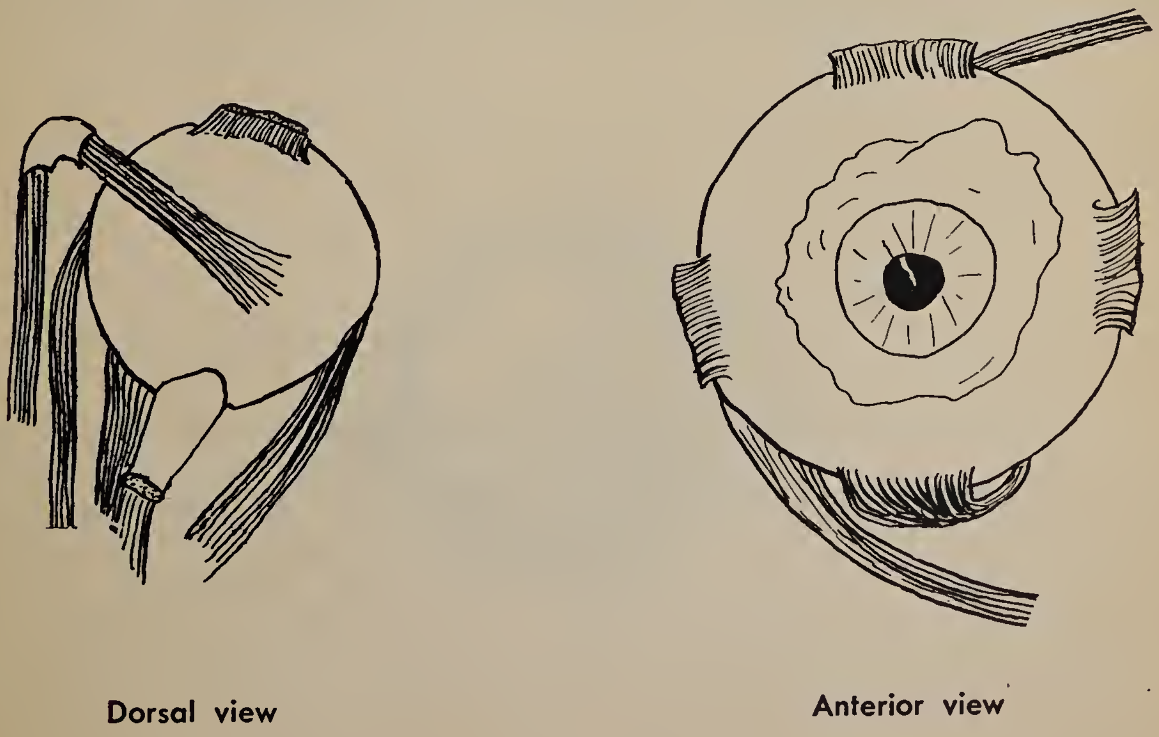


Fig. 44-4-a Diagram of the eye showing muscles

The wall of the eye contains three distinct layers. The outer layer, or "white of the eye", is the **sclera** composed of tough fibrous tissue. (e) What is its function? .... The middle layer or **choroid** coat contains a dark pigment and blood vessels. (f) What function does it perform? .... The inner coat is the **retina**, which is a nerve sheet containing the rods and cones. These are highly specialized cells which are sensitive to light. (g) What is the function of the retina? ....
The **optic nerve** extends from the back of the eyeball. (h) What important function does it perform? ....

Each of these layers is modified in the anterior portion of the eye. The sclera becomes transparent in front and forms the **cornea.** (i) What is its function? .... The choroid forms the pigmented **iris** and **ciliary body.** (j) What is the function of the iris? .... (k) the ciliary body? .... A central opening, the **pupil,** lies in the center of the iris. (l) What is the function of the pupil? .... The **crystalline lens** is suspended from the ciliary muscles. (m) What is its function? .... The retina stops just back of the lens attachment. It does not form a structure in the anterior of the eye. A transparent jellylike material, the **vitreous humor,** fills the chamber which lies back of the lens. (n) What is its function? .... The anterior chamber in front of the lens is filled with a watery fluid, the **aqueous humor.** (o) What is its function? .... Label the structures of the eye that appear in **boldface** type on the diagram of the structure of the eyeball shown in Fig. 44-4-b.

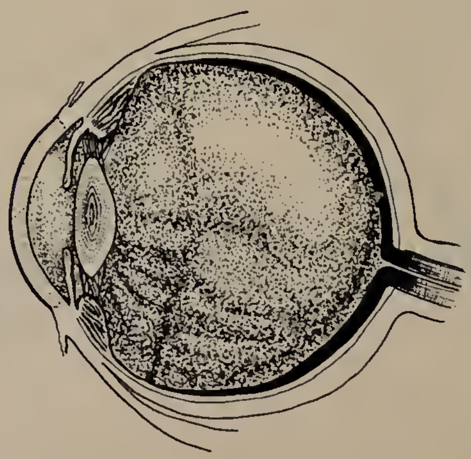


Fig. 44-4-b Diagram of the eye

A Part 2 DISSECTION OF THE EYE. This dissection is a slow process and some of the structures are very delicate. Thus, you must follow the step-by-step procedure carefully.

Materials. Cow or sheep eye; dissecting tray; hand lens; forceps; cover glass; razor blade or scalpel; dissecting needle; scissors; paper towels; newspaper.

Procedure and observations. Examine the cow or sheep eye to find the optic nerve, which will be a whitish stump protruding from the back of the eyeball. Holding the optic nerve, rotate the eyeball until two of the ocular muscles are on top. At this point an axis through the eyeball will not pass through the optic nerve because the nerve is off center. If the optic nerve pierces the eyeball to the left of the center of the posterior side, you have a right eye. (a) Which eye do you have? .... Use forceps or scissors to remove all the white, fatty tissue around the optic nerve. Examine the optic nerve. (b) Where does it enter the eyeball? .... Pull on one of the muscles attached to the eyeball. (c) How is it attached to the eye? ....

.... (d) What is the general shape of the eye? ....

....

Examine the eye and observe that it is covered with a tough, outer covering, the **sclerotic coat.** The anterior portion of the coat is called the **cornea.** (e) How does the cornea differ from the back and sides? ....

Put the eye on a wet paper towel in a dissecting tray with the posterior (back) side up. Draw the posterior view in the space provided. Label: **optic nerve: eye muscles** (see Part 1 for names); **sclerotic coat, cornea.**

Anterior view of eye (cornea removed)

Posterior view of eye

Make a small opening in the back of the eye by cutting out the optic nerve. Put a cover glass over the hole to keep the jellylike liquid, the *vitreous humor,* that fills the posterior chamber of the eye from escaping. Hold the eye up near your own eye and look through the opening you made. Move the eye in several directions until you can see the image of some object in front of it. (f) Determine whether the image is right side up or upside down. ....

....

Put the eye on a piece of wet paper towel placed in a dissecting tray so that the cornea side is up. Hold the eye firmly with one hand (do not squeeze it), and using a razor blade, make a one-eighth inch incision at the edge of the cornea. When you have cut through the cornea, a watery fluid, the *aqueous humor,* will begin to flow through the cut. Insert the point of your dissecting scissors and cut completely around the cornea. Cut slowly and carefully. Use the forceps to lift and remove the cornea.

Using the hand lens, examine the eye and locate the *circular* and *longitudinal muscle bundles* of the *iris.* Look through the *pupil* and study the *lens.* It is opaque in the preserved specimen, but is colorless and transparent in a living eye. Make a drawing of the eye with the cornea removed in the space provided. Label: **sclerotic coat; iris; pupil; lens; muscles of the lens.** Observe the circular band of fibers that attach the lens to the eye. Pull on these fibers and determine how they work. (g) Describe what happened. ....

....

Use the dissecting needle to prick a hole in the thin capsule that contains the lens. Gently tear the capsule at the edge and lift the lens out. Be sure that the fingers are moistened when you handle the lens. Place the lens on a piece of newspaper. (h) Look through the lens and determine whether it magnifies the newsprint. .... (i) Describe the shape of the lens. .... (j) Is the lens rigid? ....

Remove any membranous tissue that remains over the vitreous humor. Put the eye under a strong light or take it to the window and look into the inside of the eye. This is the view a doctor sees when he examines an eye with an ophthalmoscope.

Squeeze out the vitreous humor onto a wet paper towel. Look for a dark ring on the surface of the fluid. This is the *ciliary body,* which contains muscles that regulate the shape of the lens.

Begin at the edge of the cut in the front of the eye and make a one-half cut backwards through the side of the eye. Be careful not to touch the inside surface of the eye. Make a similar cut on the opposite side of the eye. Now turn the eye inside out. Look for the thin, delicate gray or pink membrane lining of the eye. This is the *retina,* the light-sensitive inner coat. Observe the blood vessels that line this layer. This layer also contains the specialized nerve cells known as *rods* and *cones.* If you have trouble locating the retina, it may have come loose during the dissection. If it did, you can find it as a mass attached to the *blind spot* that is located near the point at which the optic nerve pierces the eye. Submerge the eye in a bowl of water, and the retina will float free and straighten out.

Using a scalpel or razor blade, scrape the inside surface of the eye and observe the black pigment of the *choroid coat.* Continue to scrape until you have exposed the inside surface of the sclerotic coat.

A Part 3 DEMONSTRATION OF VISUAL ACCOMMODATION. The individual whose eyes are normal can see distant objects without effort when the eyes are at rest. But he has to make an effort to bring near objects into focus on the retina. This is referred to as accommodation. The ability of the eyes to accommodate is automatic and limited.

Materials. Straight pins; ruler.

Procedure and observations. The distance from the eye to the nearest object that can be focused clearly is called the *near point* of vision. To find the near point of your vision, place the hand over one eye and focus the other eye on a straight pin held at arm's length. Gradually bring the pin closer to your eye, continuing to focus on it until the pin can no longer be seen sharply. Measure the distance from your eye to the pin. (a) What is the distance? ....

This is your near point. Repeat the process with the other eye. (b) What is the distance? ....

A Part 4 LOCATING THE BLIND SPOT. There are no cones or rods in the retina at the area where the optic nerve fibers and the eyeball join. Therefore, there is no sight and this area is called the blind spot.

Materials. No materials or apparatus necessary.

Procedure and observations. Close your left eye and hold this book about twelve inches from the right eye. Look at the cross; the circle will be seen by indirect vision. Gradually, bring the book closer to the eye, or farther away from it, until you can no longer see the circle. Measure this distance. (a) Why does the circle disappear at this point? ....

.... (b) Is the distance the same for all members of the class?

.... (c) If not, explain why there is a difference. ....

....

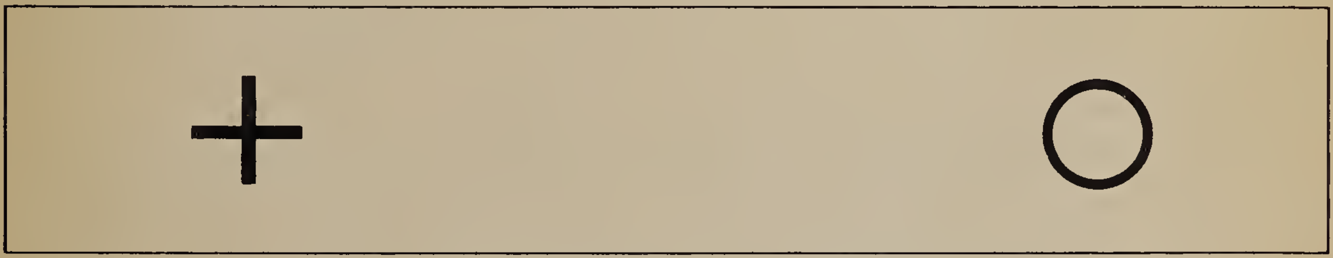


Fig. 44-4-c Chart for demonstration of blind spot

A Part 5 POSITIVE AND NEGATIVE AFTERIMAGES. The length of time required for a stimulus to produce a sensation is very short. However, the sensation lasts a great deal longer than the stimulus. For example, an electric spark may last only 1/8,000,000 of a second, yet its visible image lasts much longer. This is referred to as an afterimage. When the afterimage is the same color as the original, it is called a positive afterimage. Negative afterimages appear in the complimentary colors of the originals.

Materials. White light source; colored light source; 2 × 2-inch red-colored paper (additional colors may be used); sheet of white paper; sheet of red paper (additional colors may be used).

Procedure and observations. Adapt your eyes to the dark by sitting in a darkened room for 10 minutes. Then turn on the light for a second and look at the white bulb. Turn off the light.

(a) What kind of afterimage appeared? ....

(b) How long did it last?- ....

Now repeat the experiment substituting colored lights for the white ones. (c) Describe what happened. .... (d) What kind of afterimage appeared? .... Close your eyes and follow the afterimage. (e) Does the afterimage follow the eye movements? ....

(f) Explain your answer. ....

....

Now look intently for 20-30 seconds at a small red (other colors may be substituted) card and then at a sheet of white paper. (g) Describe what happened. ....

.... (h) What kind of afterimage appeared?

.... Look at the red card again. Then look at a sheet of red paper. (i) Describe what happened. ....

....

Focus your eyes on a colored object for 20-30 seconds. Look at a white surface for a second and then close your eyes. (j) Describe what happened. ....

.... (k) What kind of afterimage appeared?

....

Purpose To illustrate the water cycle by means of a pictorial diagram and to relate conservation problems to the cycle; to demonstrate factors involved in evaporation; to demonstrate the effect of external factors on transpiration.

Rain, sleet, and snow are forms of *precipitation.* The return of water vapor to the atmosphere is *evaporation.* Together these processes constitute the water cycle. Plants and animals are involved in the cycle as they use water for the constructive phase of metabolism and give off water during the destructive phase.

A Part 1 THE WATER CYCLE AND CONSERVATION. Following precipitation, water may run off the ground and collect in streams, ponds, and oceans. This is surface water, from which evaporation occurs. Some of the precipitation may soak into the ground and reach the water table, enter plant roots and rise through the soil to the surface, to eventually evaporate. In this Part you will label a diagram of the cycle and study conservation practices related to it.

Materials. Colored pencils; ruler.

Procedure and observations. The landscape shown in Fig. 48-1-a is a setting for the construction of a diagram of the water cycle. Movement of water will be indicated with arrows, the arrow indicating the direction of movement. The color of the arrow will classify the water as precipitation (red), surface water (blue), ground water (green), or water evaporating and returning to the atmosphere (yellow). Indicate the *kind* of water with a letter placed on the arrow, according to the description and key below the water cycle.

(a) How does loss of topsoil through erosion alter the normal water cycle? ....

....

....

(b) How is the water cycle affected by the removal of vegetation? ....

....

....

(c) Why are swamps and marshes important in a normal water cycle? ....

....

(d) Should river banks and flood plains (bottom lands) be cultivated? Explain. ....

....

(e) Why are trees and shrubs especially important in watershed regions? ....

....

(f) Explain the relation of floods to the water table. ....

....

(g) How may topsoil reduce floods and droughts? ....

....

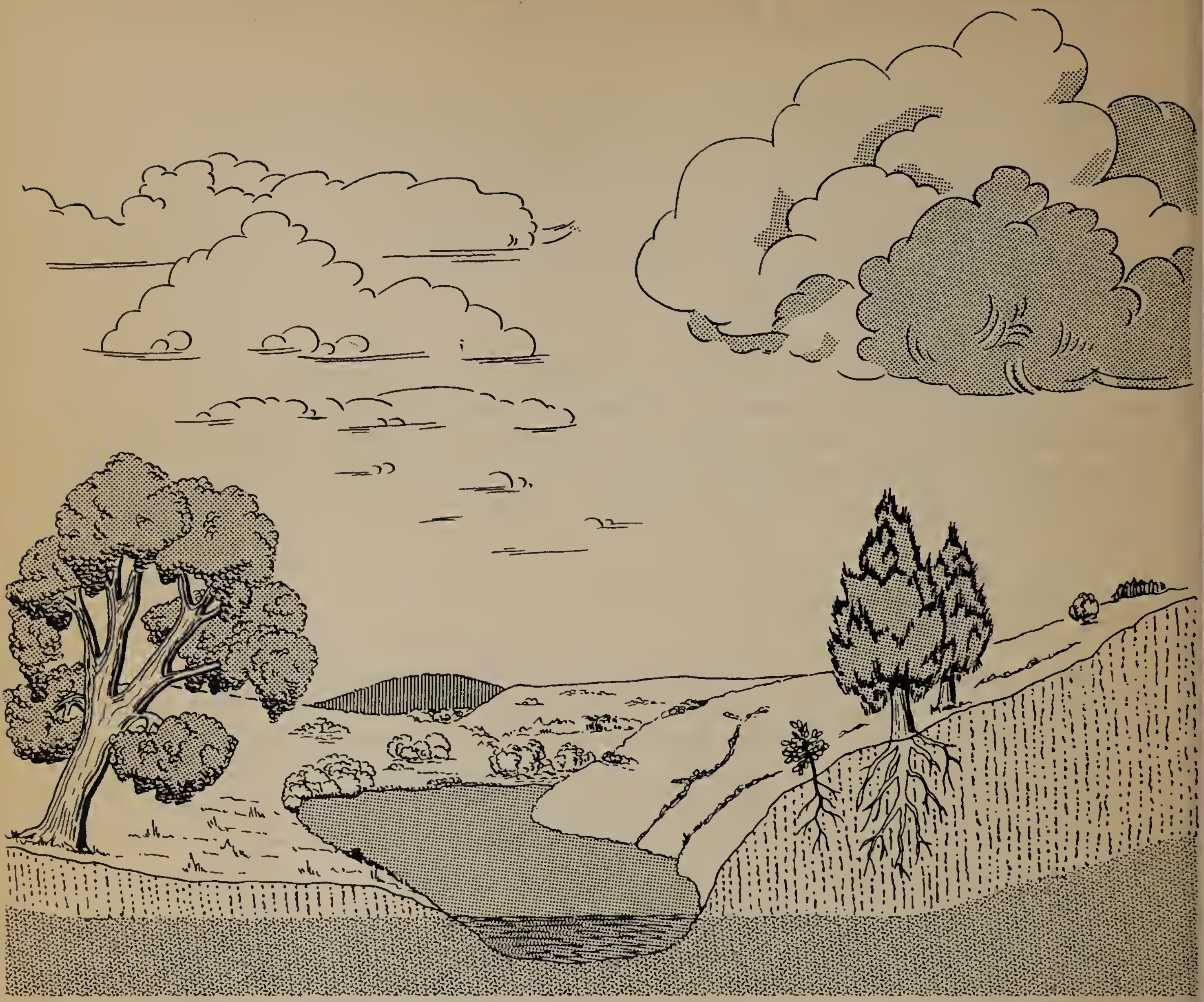


Fig. 48-1-a Water cycle

A. Precipitation from cloud to earth (red)	G. Ground water absorbed by plant roots (green)
B. Runoff water to pond (blue)	H. Water transpired from leaves to atmosphere (yellow)
C. Surface water stored in pond (blue)	I. Water evaporating from soil surface into atmosphere (yellow)
D. Ground water seeping through soil to water table (green)	J. Water evaporating from surface of pond (yellow)
E. Ground water stored at water table (green)	
F. Ground water rising through soil from water table (green)	

B Part 2 **FACTORS IN THE RATE OF EVAPORATION.** The available moisture in any region is determined by two factors: the amount of precipitation and the rate of evaporation. Thus, a shaded hillside or valley may support plants with much higher moisture requirements than those growing on the top of the hill or on the side exposed to the sun. The precipitation in the two areas is the same, but the rate of evaporation is vastly different. Evaporation rate is determined by many factors, including the relative humidity, temperature of the air, and air movement.

Materials. Shallow pan (large pie pan or baking pan) with a capacity of 500 ml; screen to cover pan; 500-ml graduated cylinder.

Procedure and observations. The effect of relative humidity and temperature on the rate of evaporation may be determined by means of a simple experiment. Select an outdoor station where a pan of water will not be disturbed for 24 hours. The station should be away from buildings or other obstructions that would interfere with air movement. Measure exactly 500 ml of water in a graduated cylinder and pour it into a shallow pan. In hot weather it may be necessary to use 1,000 ml of water. Cover the pan with a screen to prevent birds from drinking the water. After 24 hours measure the water remaining in the pan, using the graduated cylinder (it may be necessary to pour water from the pan to a beaker to fill the graduated cylinder without spilling). Record this amount and determine the amount of water evaporated in the table below. Consult your newspaper or your local weather bureau and find out the high and low relative humidity, high and low temperature, and high and low wind velocity during the 24-hour period. Record these data in the table. Repeat the procedure on five consecutive days, if possible.

	Ml water at start	*Ml water after 24 hours*	*Ml water evaporated*	*High relative humidity*	*Low relative humidity*	*High temp.*	*Low temp.*	*High wind vel.*	*Low wind vel.*
1									
2									
3									
4									
5									

The scope of the experiment may be extended by placing pans of water at various stations simultaneously. Choose different locations, such as sunny areas, shady areas, high places, low places, etc.

One of the limitations of the pan of water for measuring evaporation is the necessity of using a screen to cover it. This reduces air flow and shades the water. An ecologist often uses a device known as an atmometer, which consists of a hollow, porous clay cup or ball connected to a glass tube inserted through a stopper into a bottle containing a measured quantity of water. Water evaporates from the surface of the clay cup or ball. The amount of evaporation is determined by water loss from the bottle.

Interpretation. On the basis of your data, account for variations in the rate of water evaporation in terms of fluctuations in atmospheric conditions. ....

....

....

C Part 3 INFLUENCE OF EXTERNAL FACTORS ON THE RATE OF TRANSPIRATION FROM PLANTS

Problem. How do external factors such as light intensity, air currents, and temperature affect the rate of transpiration in plants?

Green plants play an important role in the water cycle. The amount of water lost from the leaves of plants by transpiration is much greater than the amount absorbed and used by plants in synthesis of food and other organic compounds. Light intensity, air currents, and temperature are all factors that influence the rate of transpiration. You can determine the influence of these factors on the transpiration rate by establishing the amount of water lost from leaves under classroom conditions. Then, by altering any one of these factors, you can determine its effect on transpiration rate by comparing your results with the control plant.

Suggested materials. A sprig of a plant with a firm stem such as a geranium or coleus or a woody stem such as a pine, fir, or cottonwood; 8-oz wide-mouth gas bottle or 250-ml Erlenmeyer flask; two-hole stopper to fit gas bottle or flask; 1-ml pipette graduated in tenths of milliliters; scalpel; glass-marking pencil; small electric fan.

Suggested procedure. Fill the gas bottle or flask completely full of water. Insert the 1-ml pipette graduated in tenths of milliliters in the two-hole stopper so that two to three inches extend below the stopper. Insert a sprig of geranium, coleus, or woody stem bearing leaves in the other hole so that two or three inches extend below the stopper. Submerge the cut end of the stem in a pan or beaker of water and cut off one-fourth inch of the stem under water. Quickly insert the stopper in the gas bottle or flask so that all of the air is forced out. The excess water will move into the pipette when the stopper is pressed firmly into the jar. Mark the level of water in the pipette with a glass-marking pencil and record the height of the column of water. Check the air temperature near the surface of the leaves. Record this temperature. Continue to check the water level in the pipette for two or three days, marking the level each time you check it and recording the level in your notebook. The drop of the water level indicates a loss of water from the plant. Plot your data on a graph, using the x-axis (horizontal) for time and the y-axis (vertical) for loss rate due to transpiration. This information can serve as the control against which to check the factors that influence transpiration rate.

Check the influence of air currents by producing currents with a small electric fan. Place the fan one foot, then two feet, and three feet from the plant. Before beginning your study of the influence of air currents, be sure to remove the stopper and fill the jar or flask completely, then return the stopper to the flask. Check and record the level of water in the pipette as you did for the control. Plot your data on a graph as before.

Remove the stopper from the setup and fill the flask or bottle completely and return the stopper to the flask or bottle as before. Then check the influence of light intensity on transpiration rate by placing it in an area of bright sunlight, diffuse light, or artificial light. Check each of the areas as you did for the control and record your data on a graph.

To check the effect of temperature, replace the water in the flask or bottle. Then check the various areas of the classroom for variations in temperature. The plant setup can then be placed in these areas and the water loss can be checked as for the control.

Analysis and conclusions. Summarize all of your data on a graph. Use a different color to designate the control, air currents, light intensities, and temperature. Use the x-axis (horizontal) for time and the y-axis (vertical) for water loss due to transpiration. Your data should help you to draw some important conclusions relating to the ecological distribution of plants and how vast areas of vegetation exert an influence on the climate.

Purpose To demonstrate the carbon-oxygen cycle in a closed ecosystem.

Throughout the daylight hours of a growing season, green plants are organizing glucose molecules in a series of biochemical reactions we group in the process called photosynthesis. As you recall from your study of photosynthesis, carbon dioxide and water are chemical requirements. These compounds must be available in the environment of the green plant if photosynthesis is to be carried on. Abundant as carbon and oxygen are, the supply would soon be exhausted if the rate of replenishment did not equal the rate of use. Use, replenish, and reuse—this is the concept of a cycle. Photosynthesis is the "use" phase of the carbon-oxygen cycle. The "replenishment" phase is respiration.

A Part 1 STUDY OF A CLOSED ECOSYSTEM. How large must a living community be to observe evidence of the carbon-oxygen cycle? In this Part you will construct an ecosystem small enough to carry in your pocket. By making three closed environments with important differences, you will discover to what extent organisms depend on one another in performing the processes involved in the carbon-oxygen cycle.

Materials. Living fresh-water snails; six or more vigorous terminal sprigs of elodea (or other suitable aquatic plant); three 6-in. culture tubes (test tubes); corks or stoppers to fit tubes; test-tube rack; paraffin; pan or beaker for melting paraffin; heat source; glass-marking pencil; pond or aquarium water to put in culture tubes.

Procedure and observations. Mark three 6-in. culture tubes *1*, *2*, and *3* with a glass-marking pencil. Fill each tube three-quarters full of pond water or aquarium water. Put a snail in tube 1, a snail and two or more sprigs of elodea in tube 2, and an equal amount of elodea (without a snail) in tube 3, as shown in Fig. 48-2-a. Close each tube with a cork or rubber stopper and seal it securely with paraffin. Examine the tubes daily for five consecutive days. (Unless the experiment is started on Monday, it will be necessary to take the tubes home.)

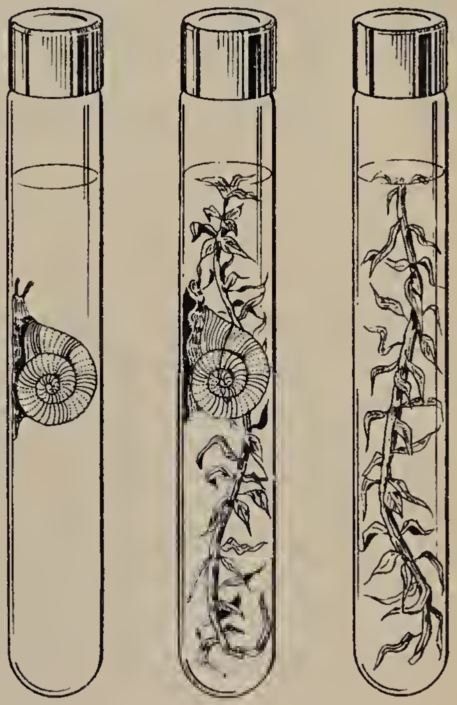


Fig. 48-2-a Closed environments

As you examine the tubes, make three observations as follows:

Is the snail alive? If so, is it active, e.g., moving on the side of the tube, feeding on elodea leaves?

Does the elodea appear to be growing, or is there evidence that it is unhealthy or dying?

What is the condition of the water? Is it clear or cloudy (turbid), indicating decomposition of plant or animal substances?

Record the observations you make each day in the table provided.

Day	*Tube 1 (snail)*	*Tube 2 (snail and elodea)*	*Tube 3 (elodea)*
1			
2			
3			
4			
5			

(a) Does your experiment indicate that an animal can survive in a closed environment without a green plant? .... (b) Explain your answer. ....

.... (c) Was the snail involved with the green plant in the carbon-oxygen cycle? .... (d) Does the green plant benefit from the presence of the snail? .... (e) Explain your answer. ....

....

(f) Can a green plant complete a carbon-oxygen cycle and continue to survive without the presence of an animal? .... (g) Explain your answer. ....

....

Plants and animals could survive in a small closed environment for a much longer period than the five days you conducted your investigation. Sooner or later, however, decomposition would need to occur. (h) Explain why this is true. ....

....

....

B Part 2 PREPARING A BALANCED AQUARIUM. Tube 2 in Part 1 was a miniature ecosystem. If the tube contained sufficient elodea to supply the oxygen and nutritional needs of the snail, the organisms could have lived in the closed environment for a long period of time. In this Part you will increase the size of the environment and add to the number of plants and animals. We will refer to this closed environment as a balanced aquarium.

Most aquaria you see stocked with tropical fish, goldfish, and other aquatic animals are not balanced. For this reason, they require aeration and artificial feeding of the animals. In a balanced aquarium, both of these requirements are supplied by the plants in the ecosystem. For this reason, care must be used to avoid overstocking the environment with animals.

Materials. Small fish (1-2 in. long); several fresh-water snails; various aquatic animals, including a small crayfish, tadpole, salamander, water beetle, water boatman, dragonfly nymph, helgrammite, etc.; water plants, including elodea, vallisneria, etc.; large wide-mouth jar (1-3 gal); clean coarse sand; pond or aquarium water.

Procedure and observations. Use either a large wide-mouth jar with a stopper or a screw cap or a 1-3 gal aquarium tank for setting up your balanced aquarium. Cover the bottom of the jar with one to two inches of thoroughly washed coarse sand. Coarse sand is necessary to aerate the roots of the plants. Fill the jar to within two inches of the top with pond water or aged water taken from another aquarium. Root several strands of vigorously growing elodea or vallisneria in the sand (these and other rooted aquarium plants are available in aquarium stores, pet stores, and many department stores). Use at least one fish, following the general rules of one inch of fish to one gallon of water. Small goldfish, catfish, or carp are ideal residents in a balanced aquarium. Add several fresh-water snails, one or two small crayfish, a tadpole, and several aquatic insects, if available. After stocking the aquarium, close the top securely. If an aquarium tank is used, it may be closed with a glass plate and sealed around the edges. If possible, put the aquarium in a north window. If a sunny window is used, cover the exposed side with a sheet of green cellophane to reduce the light and prevent excessive growth of algae. If sufficient light is not available and the plants begin to lose their green color, put an electric lamp with a 75-watt or 100-watt bulb over the aquarium for several hours each day.

Examine the aquarium closely for several days after setting it up. If the fish, tadpoles, and any other animals come to the surface for oxygen, it will be necessary to remove some of the animals or increase the number of plants. Provision must be made, also, for including a plant or animal diet suitable to the fish you use.

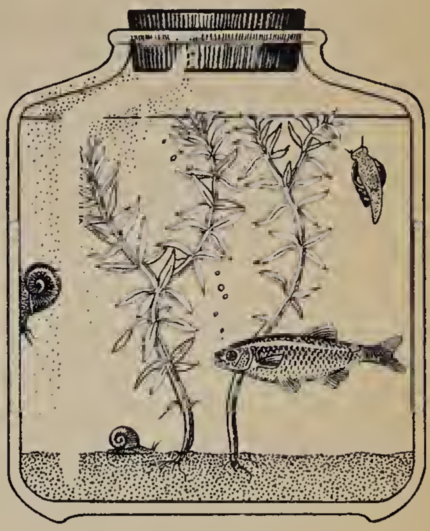


Fig. 48-2-b
Balanced aquarium

When a balanced condition is reached and the plant and animal populations are thriving in the closed environment, prepare a data summary of the organisms used in the following table.

Type of container	*Water capacity*	*Number and kinds of plants*	*Number and kinds of animals*

Interpretation. From your observations of the balanced aquarium you prepared, discuss the biological principles of a closed environment. ....

....

....

....

....

Purpose To illustrate nutritional relationships and interdependence of organisms in a local biotic community and to study symbiotic relationships in animals.

The influence of living things on one another is as important as the effect of physical factors. Many of the biotic relationships involve food chains. One form of life feeds on another and the food source upon which all forms are directly or indirectly dependent is the green plant. In this Investigation you will study the various organisms involved in food chains in a particular biotic community and determine the relative number of organisms involved at each level of a food pyramid that you construct.

A Part 1 ILLUSTRATION OF A FOOD CHAIN. The operation of a food chain may be observed in any biotic community of your area. Autotrophic plants use the chemical supplies of the earth and atmosphere and synthesize organic nutrients which are consumed by plant-eating animals. These animals in turn become the prey of other animals as matter passes from one organism to another in a food chain. Such a chain may be shown graphically in pictorial form.

Materials. No materials or apparatus required.

Procedure and observations. Select a biotic community in your area, such as a lake, a pond, a bog, an open field, a forest, or a desert. In the space provided illustrate a green plant that grows in the community as a basic food producer in the food chain. Next show an herbivorous animal that commonly feeds on the plant. Continue the food chain by including several predators. Your chain will probably end with a large carnivore. In selecting your organisms and illustrating them, choose plants and animals that share the environment and are involved in a food chain in nature. Name the biotic community you have selected and each organism shown. If possible, base your food chain on actual observations you have made in the field.

Food chain in a .... community

All the organisms involved in a food chain require energy to support their processes. (a) What is the original source of this energy?

(b) Why is the autotrophic (green) plant essential in a food chain?

The food chain you have illustrated is actually a segment of a cycle of matter. The matter represented in the carnivore with which you ended the food chain will eventually be changed to forms that can be used by an autotrophic plant as the cycle repeats. (c) Why are scavengers important in a food chain?

(d) What is the role of the decomposers in a food chain?

A food chain also involves energy transfer. (e) Does energy cycle?

(f) Explain your answer.

A Part 2 **CONSTRUCTION OF A FOOD PYRAMID.** Food chains are often represented as a pyramid, with the food producers forming the base and the top carnivore at the apex. In a food chain, each organism consumed becomes a part of the substance of the consumer. However, only a part of the matter consumed is represented in the organism that consumed it. For example, only a portion of the plant material eaten by a caterpillar is represented in the mass of the caterpillar. If a bird in turn eats caterpillars, the mass of the bird is considerably less than the mass of the caterpillars eaten. Thus, each step in the food chain represents a reduction in the total amount of organic material, or biomass. This may be shown graphically in a food pyramid.

Materials. No materials or apparatus required.

Procedure and observations. Examine the organisms involved in the food chain you illustrated in Part 1. Construct a pyramid in the space provided using the steps illustrated in your food chain. The base of the pyramid represents the soil and atmosphere. Autotrophic plants, herbivores, and carnivores are represented in various levels of the pyramid. In constructing these levels from your food chain, etimate the relative number of each group found in the biotic community you selected.

(a) Why is the biomass reduced in each level of a food pyramid?

(b) As illustrated in a food pyramid, which group of organisms is most numerous in a biotic community?

(c) Which group is least numerous?

A food pyramid

(d) Where is the most food energy in a food pyramid? ....

(e) What factors influence the length of food chains in various regions? ....

C Part 3 STUDY OF FROG PARASITES

Problem. What are some organisms that are parasitic on the frog?

Green plants produce food. Herbivores consume plants and carnivores consume other animals. In another kind of nutritional relationship called symbiosis, individuals live in direct association with one another. Parasitism is a common form of symbiosis. In parasitism the parasite benefits while the host is harmed. Every free-living organism appears to have its parasites. Many of them have more than one kind of parasite. The common frog can be used to study parasites because it often has them on its skin, between the skin and muscle, and in various tissues in its body.

Suggested materials. Live frogs; microscope; slides; cover glasses; large-mouth jar or plastic bag for etherizing the frogs; cotton; dissecting tray; scalpel; scissors; forceps; dissecting needles; straight pins; Petri dishes; ethyl ether; 0.7% saline solution; methylene blue; references in parasitology.

Suggested procedure. Examine the frog's skin to look for ticks. Study the structure of the ticks under the microscope, noting the type of mouthparts and presence or absence of appendages. Make a sketch of the ticks.

Etherize the frog by putting it into a large-mouth jar or plastic bag containing cotton soaked in ethyl ether. When the frog is limp, remove it from the container and place it ventral side up in a dissecting tray. Pin it to the tray by stretching its legs and inserting a pin in the front and hind feet.

In examining all of the parasites you find in the frog, prepare a slide by putting a few drops of saline solution on the slide, then add the tissue containing the parasites. Put a few drops of methylene blue on the tissue to stain the parasites for better study. Cover with a cover glass and examine under low or high power, depending on the size of the parasite. Make a drawing of all the parasites you find. Be sure to note whether the parasite is attached by means of special organs or free. If free, note the means of locomotion. Determine the presence or absence of a mouth, digestive tube, and anus. Look for reproductive organs and determine whether the sexes are separate or the animal is an hermaphrodite.

Cut off one of the fingers and collect a drop of blood on a microscope slide. Cover with a cover glass and examine under the high power of the microscope. Search among the red blood cells for small worm-shaped organisms, the embryonic nematodes. If these are present in the blood, you can expect to find some of the adults between the skin and muscle wall. Make a longitudinal cut along the trunk with your scissors. Look for the adults between the skin and muscle wall, in the abdominal and axillary regions, or in the thoracic and abdominal cavities.

Remove the entire contents of the thoracic and abdominal cavities. Separate the organs and put them in individual Petri dishes containing a small amount of saline solution. Pull the lung and liver tissue apart with your dissecting needles. Examine with the naked eye for the presence of roundworms and flatworms. Then examine some of the tissue under the microscope for other parasites.

Cut open the stomach and intestine and examine the contents with the naked eye and with a microscope for the presence of parasites. Examine the contents of the urinary bladder and the bladder tissue with the naked eye and with a microscope for flatworms and roundworms. Cut open the rectum and mount bits of the fecal wastes for microscopic examination.

If you wish to further your study of symbiosis, you may examine the intestine of the termite for cellulose-digesting flagellates that live there, or examine the seminal vesicles of the earthworm for protozoans. You might also collect materials from ponds and look for green hydras or paramecia that have green alga living with them in a symbiotic relationship.

Analysis and conclusions. Summarize your findings in the form of drawings and tables. You should be able to reach conclusions as to how harmful the frog parasites are to the host.

Purpose To study two habitats and to observe the factors that enable organisms to live there.

The habitat of an organism is the place where it lives. The type of soil, temperature, available water, and light conditions are some physical factors that limit the organisms that can live in a given habitat. The other living things present also determine the nature of the habitat.

B Part 1 COMPARATIVE STUDY OF TWO HABITATS. Your study of habitats will be limited by the communities in which you live, but you will not have to travel far in your community to discover that habitats vary considerably as to their physical and biotic factors.

Materials. Thermometer (weather type); photographic light meter (if available); plant press; large magazine; Hydrion paper; identification keys for plants and animals.

Procedure and observations. Select two totally different environments for your study. One should be open, such as a field or clearing. The other should be protected, such as a woods or a deep ravine. Conduct your studies on the same day and, as nearly as possible, under the same conditions.

Determine the temperature in the first environment and record it in your notes. If a photographic light meter is available, take a reading and record it. If you do not have a light meter, judge the light as bright, somewhat reduced, or very reduced. Judge the air movement as windy, slightly breezy, or still. Take a sample of the soil and put it in a bottle. Examine it closely and, if possible, classify it as humus, loam, sand, clay, or a mixture of two of these types. Record the type of soil. It may be necessary to examine the soil further in the laboratory to determine the type. Using Hydrion paper, determine the pH of the soil. Record the pH in the table provided.

Judge the soil moisture as wet, damp, dry, or very dry. Study the plants growing in the region. Collect specimens of the different plants you find in an area of about a half acre (be careful to avoid poison ivy or other poisonous plants). Press the plants in a plant press or large magazine. Collect a leaf only from the trees, shrubs, or vines. You can identify these later by using various keys and with the help of your teacher. Observe any animals you see in the environment. Include birds and mammals, snails, insects, and any other forms you see and can identify.

Classify the organisms as to whether they are autotrophs, (food producers) or heterotrophs (food consumers). If they are heterotrophs, further divide them into herbivores and carnivores, indicating any special requirements and the major food consumed by the heterotrophs.

(a) If there were different groups of producers in the two habitats you studied, which group contributed most to food production in one habitat? ....

(b) the second habitat? .... (c) Which consumers (first and second-level) were found in one habitat? ....

(d) the second habitat? .... (e) Cite any evidence of predator-prey or parasite-host relationships, mutualism, commensalism, or scavengers in one habitat. .... (f) the second habitat. ....

Kind of Environment		
Physical factors		
Temperature		
Topography		
Light conditions		
Air currents		
Soil type		
Soil pH		
Soil moisture		

Kind of Environment				
Biotic factors	*Special requirements*	*Major food*	*Special requirements*	*Major food*
Autotrophs (food producers)				
Heterotrophs (food consumers) Herbivores				
Carnivores				

Interpretation. On the basis of the data you collected, cite any evidence you found that physical factors influence the type of organisms that can occupy a habitat. In addition, discuss the interrelationships of the species of organisms you found. ....

....

....

....

Purpose To become familiar with organisms that inhabit the soil by studying soil samples from different areas.

There are literally millions of organisms in each gram of soil. Besides the bacteria, which act as decomposers and transformers, there are usually various algae and fungi and there may be true plants and multicellular animals. Although the bacteria of decay are constantly at work decomposing other soil organisms, some organic substances do not degrade easily. As a result soil may contain a supply of organic matter, called humus, which makes it porous as well as fertile.

B Part 1 COMPARATIVE STUDY OF THE FAUNA IN VARIOUS SOILS. To study the organisms that live in the soil, it is necessary to collect samples from the various areas and take them to the laboratory for examination. Since physical factors such as soil conditions influence the type of organisms found in a community, you should select two or more types of soil communities for study.

Materials. Dissecting microscope; hand lens; meter stick; string; 4 stakes; shovel; shoe boxes or other containers for soil samples; Berlese funnel or equipment to make one, as shown in Fig. 49-3-a; jar; goose-necked lamp or other light source; alcohol.

Procedure and observations. Select soil from two or more areas such as a woods, open field, or garden for your study. The procedure will be the same for the study of the various soils. Mark off a plot 30 cm (about one foot) square and place stakes at the four corners. Now connect the four corners with string. This defines the area you will study.

Insert a shovel and make a cut around the square to a depth of 5 cm (about two inches). Cut under the square and put it into a container to transport it to your laboratory. (a) How many square centimeters of soil are there in this sample? ....

(b) How many cubic centimeters? ....

Remove a portion of the soil from this section and put it on a sheet of white paper. Separate the soil particles and examine them with a hand lens. Collect all the living organisms you can detect. Classify these as to whether they are insect larvae, pupae, or adults, worms, etc. Continue until you have examined all of the soil in each sample.

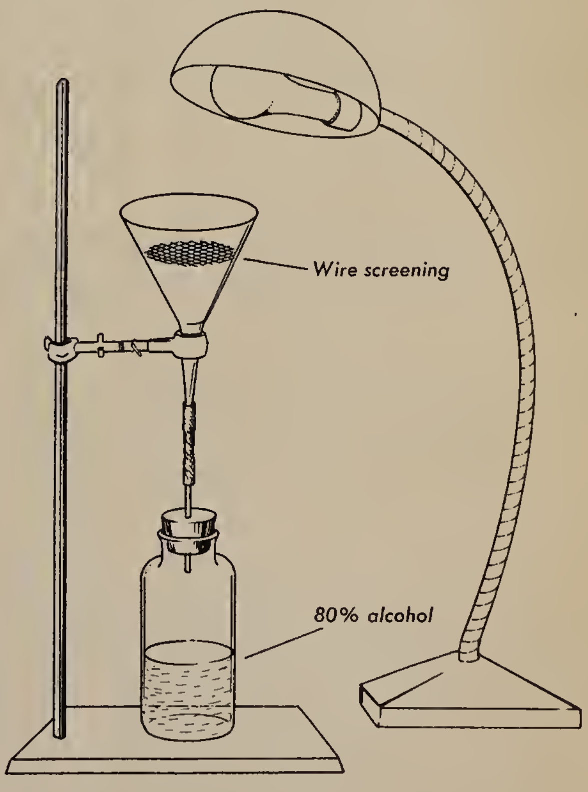


Fig. 49-3-a Berlese funnel apparatus

Many more organisms can be observed by setting up the apparatus shown in Fig. 49-3-a. Put the contents of one box into the funnel. Be sure to identify the box. If enough equipment is available, you can set up as many funnels as you have soil samples. The heat and light from the lamp will drive the remaining organisms down where they will fall through the neck of the funnel and into the jar of alcohol. The apparatus may be left overnight. Classify as many of these organisms as you can. Prepare a table on a separate sheet of paper summarizing your soil population analysis. Tabulate those you cannot identify as *unclassified.* Construct other columns for as many groups as you can establish and identify.

Determine the type of soil for each sample, the volume, number of organisms present, number of organisms per square centimeter, and number of organisms per cubic centimeter, and record in the table provided.

Sample number	*Type of soil*	*Volume*	*Number of organisms*	*Number of organisms per square centimeter*	*Number of organisms per cubic centimeter*
1					
2					
3					

Interpretation. On the basis of the data you collected, give possible reasons to account for the variation in the kind and density of organisms in the soil samples you examined. ....

....

....

Succession

51-1

Purpose To study succession in a microcosm and to study evidences of succession in several communities.

Most communities of plants and animals tend to change. A group of organisms is replaced by a second; the second may be replaced by a third. Each new organism that gains supremacy alters the environment and provides conditions that favor other species. Thus, populations may change from hour to hour and day to day. Some successions, such as changing organisms in a temporary rain puddle, occur on a very small scale. Such a succession is usually over in a matter of days. Other successions, such as a change from bare ground to mature forest, may take a century or more.

B Part 1 SUCCESSION OF PROTISTS AND ASSOCIATED FORMS IN A FRESH-WATER CULTURE. The protozoan successions that occur in temporary rain puddles can be simulated in the laboratory by collecting samples of pond water and examining them periodically for organisms that have assumed supremacy.

Materials. Five jars to collect pond water; cultures of pond water; microscope; Hydrion paper; pipette; centigrade thermometer; slide; cover glass; light meter (if available); camera (if available).

Procedure and observations. Select a stream, pond, or lake from which you can collect your water cultures. Fill five quart jars three-fourths full of water containing some plant material. Some cultures should contain floating vegetation such as duckweed, others should contain algae only, and one culture should contain some submerged vegetation and dead plant material. This will create a different environment for each culture.

Place the cultures in an area of the laboratory where each will receive the same amount of light. If you have a light meter, check the light at various times of the day. (a) What is the variation in foot candles of light available to the cultures? ....

(b) Why is light important in the study? ....

Using a centigrade thermometer, check the temperature of the culture each day that you examine the water. (c) Why is temperature an important factor in succession in the jar?

....

Using Hydrion paper, check the pH of the medium each day of the examination and draw a line graph of the changes. (d) Why is the pH of the medium related to succession? ....

....

(e) What factor is responsible for the change in pH? ....

Examination of the cultures should be made on alternate days for a period of three weeks. Since organisms occur at all levels of the culture, remove a drop from the surface, a drop from approximately 4 cm from the surface and one from the bottom for study. Study each drop of water under the low power of the microscope and determine what organisms are present.

In the five drops taken from the surface, count the number of each species present. Determine the average number of each species in the five drops. Set up a chart on which to record these averages. The chart should include such groups as green protists, nongreen protists, rotifers, crustaceans, annelids, gastrotrichs, and others, if you find them.

You might make additional records of your findings by taking photomicrographs of the organisms and drawing each of the species you study.

(f) What type of organisms did you find on the first day of examination? ....
.... (g) How long did these remain the dominant organisms?
.... (h) Describe any changes that occur in the water. ....
.... (i) What caused
the changes in the water? ....
(j) What organisms assumed dominance after the water became turbid? ....
.... (k) Explain why these organisms became dominant.
.... (l) Explain why the cultures became
clear at the end of the experiment. ....
.... (m) What organisms
followed *Colpoda* and *Colpodium* in the succession? ....
(n) What other organisms occurred in the succession? ....
....
(o) When did the rotifers and gastrotrichs appear? ....
.... (p) What factors govern
the succession in the water cultures? ....

Interpretation. On the basis of your results, summarize the course of succession in a fresh-water culture and discuss the factors that govern it. ....
....
....

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